PROGRESS REPORT -- 1995/1996

CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

AGRICULTURAL RESEARCH SERVICE

U.S. DEPARTMENT OF AGRICULTURE

P.O. Box 14565, Gainesville, FL 32604
1600/1700 SW 23rd Drive, Gainesville, FL 32608

352-374-5860 - Phone
352-374-5852 - Fax

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This report includes results of research in progress. It is not intended for publication, and should not be referred to in literature citations.

In May, 1996 the Insect Attractants, Behavior and Basic Biology Research Laboratory and the Medical and Veterinary Entomology Research Laboratory were merged into a single research institute with the title, **"Center for Medical, Agricultural and Veterinary Entomology"** (CMAVE). This is the first ARS-USDA research center devoted exclusively to entomology. The overall goal of the research program is to develop integrated management technologies and strategies for insects and other arthropods of medical, agricultural and veterinary importance. This report provides abstracts of research in progress for the information of our cooperators and other interested parties. It is not intended for citation in any publication. Reprints of published articles may be obtained by writing the individual authors at the Center address.

MISSION STATEMENT

The mission statement is as follows: The Center will conduct research on insects of agricultural, medical and veterinary importance with the goal of achieving control of pest species through the development of environmentally acceptable approaches. Emphasis is placed on developing components and systems for integrated pest management, based upon an understanding of the behavior, physiology and ecology of pest species. Sensitive detection devices that employ semio-chemicals and electronic technology will provide the means for early intervention. Investigations will lead to biological control based on parasites, predators and microbes, and thus provide alternative biorational tools for managing populations of pest species. Special attention is focused on insect pests of field and horticultural crops, stored products and on arthropod pests of medical and veterinary importance. Protection of humans from arthropods of medical importance is a continuing priority. The scope of the Center's research is national and international and impacts agricultural production, postharvest storage and transport of agricultural commodities, and protection from household and disease carrying arthropods. Research is conducted to meet the needs of state and federal regulatory agencies, the Department of Defense, industry, universities, growers, commodity groups and the public at large.

STAFF AND ORGANIZATIONAL CHANGES

There were a number of changes in the organization and scientific assignments as a result of the creation of CMAVE. These are reflected in the enclosed organizational chart. In summary, one research unit, Postharvest and Bioregulation, is devoted exclusively to stored product insects; the Chemistry Research Unit and the Behavior and Biocontrol Research Unit focus on crop pests, while the Mosquito and Fly Research Unit and the Imported Fire Ant and Household Insects Unit conduct research on insects of medical and veterinary interest. As part of this reorganization Drs. S. Ferkovich, P. Greany and A. Handler transferred into the Behavior and Biocontrol Unit; Dr. D. Focks transferred into the Imported Fire Ant & Household Insects Unit; and Dr. G. Mount transferred into the Mosquito and Fly Unit. In addition, there were several departures from Gainesville by CMAVE staff. Dr. D. Haile has transferred to the ARS Screwworm Project in Panama, and Dr. S. Miller has resigned from the USDA to pursue another career. In addition Dr. P. Landolt is now located at the ARS Laboratory in Yakima, Washington. Also, Major T. Carpenter transferred to another DoD installation, and CAPT H. Bolton has replaced him as Research Liaison Officer at CMAVE.

October, 1996

PERSONNEL AND UNITS OF THE CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

Telephone (352) 374-5860 Fax (352) 374-5852

Herbert Oberlander, Center Director

ADMINISTRATIVE STAFF

Fax: 352-374-5852

H. Oberlander, Center Director; 374-5700
B. Bayer, Secretary; 374-5901
M. Robinson, Office Automation Clerk
C. Godfrey, Administrative Officer; 374-5861
B. Castro, Office Automation Clerk
E.S. Holmes, Purchasing Officer
R.B. Nettles, Supply Clerk
E.T. White, Office Automation Clerk
J.D. Ohlson, Air Cond./Refrig. Mechanic
T.L. Henry, Custodial Worker
L.T. Kelly, Custodial Worker
W.L. Munroe, Carpenter
E. Paulsen, Laborer*
J.C. Teele, Air Cond./Refrig. Mechanic
A. Woods, Custodial Worker
S.G. Straub, Safety & Occupational Health Specialist
J. Sapp, Data Entry & Technical Support*
J. Sullivan, Accounting Technician
D.W. Taylor, Purchasing Officer

LIAISON OFFICER

Herbert T. Bolton, CAPT, U.S. Navy

BEHAVIOR AND BIOCONTROL

Fax: 352-374-5804

E. R. Mitchell, Research Leader; 374-5710
V. Malcolm, Secretary; 374-5726
W. Copeland, Biological Technician
J. Leach, Biological Technician
J. Gillett, Biological Aid
R. Furlong, Biological Technician
J. Okine, Entomologist*
G. Hu, Entomologist*
D. Sieglaff, Biological Technician*
K. Gallagher, Biological Aid
J. Coleman, Student Aid*
S. Lovvorn, Biological Aid
O. Molina, Laboratory Technician*
A. Harari, Visiting Scientist
S. M. Ferkovich, Research Entomologist
C. R. Dillard, Biological Technician
R.D. Miller, Biological Technician
P. D. Greany, Research Entomologist
T. Nguyen, Biological Technician
E. Oelfke, Student Aid*
A. M. Handler, Research Geneticist
R. Harrell, Research Associate*
G. P. Whitmer, Biological Technician
M.S. Mayer, Research Entomologist
J. E. Sharp, Biological Technician
J. Sivinski, Research Entomologist
G. Posey, Biological Technician
A. Eitam, Graduate Student
E. Menezes, Visiting Scientist
Elen Menezes, Biological Technician*
R. Meagher, Research Associate

CHEMISTRY

Fax: 352-374-5707

J.H. Tumlinson, Research Leader; 374-5730
P. Zelonka, Secretary; 374-5731
H. Alborn, Visiting Scientist
P. Paré, Research Plant Physiologist
U. Röse, Visiting Graduate Student
M. M. Brennan, Physical Science Technician
S. Sharp, Student Aid
M. Donohue, Student Aid
P.E.A. Teal, Research Physiologist
J. Meredith, Research Physiologist
S. Eagan, Student Aid
R. R. Heath, Research Chemist
N. Epsky, Research Entomologist
B.D. Dueben, Physical Science Technician
A.T. Proveaux, Chemist/Spectroscopist
R. Golub, Laboratory Technician
L. Gunasekara, Technical Assistant
B. Talton, Student Aid

IMPORTED FIRE ANT & HOUSEHOLD INSECTS

Fax: 352-374-5818

R. J. Brenner, Research Leader; 374-5855
M. R. Martin, Secretary; 374-5903
C. Castro, OA Clerk
M. Ramos, OA Clerk
A. Akers, Graduate Assistant*
D. Harrison, Biological Technician
E. Mena, Biological Technician
D. Milne, Biological Technician
T. Parker, Engineering Technician
M. Romero, Engineering Technician

D. A. Focks, Research Entomologist
E. Daniels, Computer Programmer
M. McKenzie, Computer Assistant
*P. G. Koehler, Research Entomologist**
*N. Al-Kattan, Graduate Assistant**
*H. Arroyo-Sanchez, Graduate Assistant**
*B. Carpenter, Biological Scientist**
*J. Cooksey, Graduate Assistant**
*D. Godwin, Biological Aide**
*W. Grush, Graduate Assistant**
*G. Marshall, Biological Scientist**
*D. Miller, Graduate Assistant**
*J. Morehouse, Graduate Assistant**
M. Korzoukhine, Visiting Scientist
R. S. Patterson, Research Entomol. (Retired)
S. D. Porter, Research Entomologist
T. Krueger, Biological Technician
S. M. Valles, Research Entomologist
V. Johnson, Biological Science Aide
C. Strong, Biological Technician
R. K. Vander Meer, Research Chemist
L. Davis Jr., Biological Technician
M. Hosack, Chemist (0.5)
D. F. Williams, Lead Scientist
J. Burley, Biological Aide
D. Hall, Biological Technician
E. Helms, Biological Science Lab Technician
G. Knue, Biological Technician
*R. Levy, Biological Aide**
*J. Petti, Graduate Assistant**
D. P. Wojcik, Research Entomologist
B. Mayfield, Biological Science Aide
J. Sullenger, Agric. Research Technician

MOSQUITO AND FLY
Fax: 352-374-5922

D. R. Barnard, Research Leader; 374-5930
E. P. Rountree, Secretary; 374-5931
K. McRae, OA Clerk
J. Jackson, Biological Technician
*V. Droit, Biological Aide**
K. Posey, Biological Technician
D. Smith, Biological Technician
J. J. Becnel, Research Entomologist
M. Johnson, Biological Technician
T. Jacques, Biological Science Aide
D. A. Carlson, Research Chemist
U. Bernier, Chemistry Technician
M. Hosack, Chemist (0.5)
*L. Okedi, Graduate Student**
A. F. Cockburn, Research Geneticist
*B. Moser, Biological Science Technician**
T. Fukuda, Microbiologist
O. Willis, Biological Technician
C. J. Geden, Research Entomologist
C. Gerhardt, Biological Science Aide
J. Thomas, Biological Technician
J. A. Hogsette, Research Entomologist
N. Lockhart, Biological Science Aide
F. Washington, Biological Technician
D. L. Kline, Research Entomologist
*D. Burkett, Graduate Student**
H. McKeithen, Biological Technician
G. A. Mount, Research Entomologist
*J. Reinert, Research Entomologist**
J. A. Seawright, Research Entomologist
M. Falkner, Biological Technician
*K. Kangas, Biological Technician**
A. H. Undeen, Research Entomologist
S. White, Biological Technician
H. Scheer, Biological Science Aide
*R. Xue, Assistant in Entomology**

POSTHARVEST AND BIOREGULATION
Fax: 352-374-5733

H. Oberlander, Research Leader; 374-5700
P. Egger, Secretary; 374-5703
C. E. Leach, Entomologist
R. T. Arbogast, Research Entomologist
D. Weaver, Research Entomologist
L. Wojcik, Biological Technician
*L. McCandless, Biological Technican**
P. Kendra, Biological Technician
*S. Chini, Biological Technician**
R. W. Mankin, Research Entomologist
E. Foreman, Physical Science Technician
B. A. Weaver, Biological Technician
P. D. Shirk, Research Physiologist
M. Hapner, Biological Technician
*J. Goodrich, Student Aid**
*O. P. Perera, Postdoctoral Scientist**
D. Shuman, Research Electrical Engineer
*R. Johnson, Computer Programmer**
*N. Nasah, Engineering Technician**
*P. Sun, Graduate Student**
*K. Coggins, Engineering Student**
D. L. Silhacek, Research Chemist
C. L. Murphy, Biological Technician
S. Dyby, Research Associate
S. Parisek, Student Aid
J. Williams, Student Aid
H. Burnside, Biological Technician
A. Sloan, Student Aid

CROP INSECT REARING STAFF

E. R. Mitchell, Supervisor
F. Adams, Biological Technician
*G. Encabo, Laboratory Technician**
N. Lowman, Laboratory Technician
*J. Gil, Laboratory Technician**

**University of Florida Personnel*

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EFFECT OF HABITAT TYPES ON POPULATION DENSITIES AND PARASITISM OF DIAMONDBACK MOTH LARVAE IN CABBAGE FIELDS

G.Y. Hu[1], E.R. Mitchell and J.S. Okine[1]

Objective: To evaluate the effects of surrounding non-host plant neighbors on the population density of diamondback moth (DBM) larvae on cabbage plants, larval parasitism, and pest-caused damage to cabbage.

Methods: Cabbage plants in five commercial fields near Bunnell, Flagler County, FL, were sampled weekly for DBM larvae in spring 1995. Five sampling zones across each field were arranged as follows: two each along an end, one in the middle, and two each between the middle and end (1/4 length of the field). Collected larvae were dissected in the laboratory for parasitoids. At harvest, 13 mature consecutive cabbage heads larger than 15.2 cm diam were rated for damage by using the scales (1-6) developed by Greene et al. (1995) and modified by Leibee et al. (1995). Data were transformed by log (n + 1) to meet the assumptions of statistical tests and analyzed using ANOVA.

Results: DBM larvae were more abundant at the ends of fields adjacent to weed-filled drainage ditches than at field ends abutting wooded swamp areas. There was no significant difference in the numbers of DBM larvae on cabbage plants on the edge of fields next to other cabbage fields or at sites located towards the interior of the fields. Three of five fields sampled showed a spread of DBM from the ends next to drainage ditches inward up to 1/4 the length of the fields. Cabbage heads rated for damage by DBM larvae at harvest showed a distribution pattern similar to that observed for DBM larvae. Parasitism of DBM larvae were not significantly different between the ends and interior sites. These results suggest that DBM first invaded cabbage fields from outside areas, and that more DBM spread to the interior of the fields from adjacent open, weed-filled ditches than from bordering wooded and bushy areas. Strengthened control of this pest along field edges and surrounding habitats may prevent DBM populations from build-up in the fields.

Plans: Further studies will be conducted to clarify what weed species serve as the non-host plants of DBM around cabbage fields.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

HOST-AGE DISCRIMINATION OF *DIADEGMA INSULARE* UNDER LABORATORY CONDITIONS

G.Y. Hu[1], E.R. Mitchell and J.S. Okine[1]

Objective: To evaluate which instar of the diamondback moth was most likely attacked and successfully parasitized by the parasitoid *Diadegma insulare* (Cresson) under laboratory conditions.

Methods: The parasitoids were collected from collards transplanted next to cabbage fields near Bunnell, Flagler County, Florida, May 1995, and reared on DBM larvae fed on artificial agar diet. Colonies of parasitoids and hosts were maintained under 21°C, 70-80 RH, and 14:10 (L:D). Observations were made from 1100 to 1500 hours when females are the most active (Idris 1995). Each test contained one 3-d old mated female *D. insulare* and 20 DBM larvae (5 for each instar). The wasps and larvae were confined in a 13.7 cm diam X 1.3 cm high petri dish. Parasitization rates were determined by dissecting the attacked larvae.

Results: An average of 6.9 ± 5.5 (SD) 1st and 2nd instars were stung, but 16.5 ± 8.1 3rd and 4th instars were stung by each *D. insulare* ($t = 3.68$, $df = 22$, $P < 0.01$). The parasitization rates were 0.5 ± 0.9, 2.5 ± 1.2, 2.2 ± 1.8, and 2.2 ± 1.2 for the 1st, 2nd, 3rd, and 4th instar of DBM larvae, respectively. Significant difference were shown between the 1st and the other instars ($F = 19.82$; $df = 3, 40$; $P < 0.05$), but no significant differences were shown among the 2nd, 3rd, and 4th instars. Our results suggest that first instar DBM larvae were not appropriate hosts for *D. insulare*.

Plans: No further studies have been planned.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

CONCEP PHEROMONE DISPENSERS FOR DIAMONDBACK MOTH: EFFECTS ON TRAP CAPTURE OF MALES AND MATING OF SENTINEL FEMALES IN CABBAGE

E.R. Mitchell

Objective: To evaluate Concep pheromone dispensers for disrupting mating of diamondback moth (DBM) in cabbage.

Methods: The test was set out in a commercial cabbage field near Bunnell, Flagler County, FL, from 27 February-01 May. The treatments were arranged in an west-east line along the southern edge of a 30 ac cabbage field. The test field was bordered on the south by older cabbage (30 ac), north and west by paved county roads, and east by potatoes. Both cabbage fields were sprayed with pesticides every 7-10 days throughout the test period. Two replicates of each treatment, designated DBM-A and DBM-B, were arranged alternately across the field. Each plot was 0.5 ac in size, and the distance between blocks was 150 ft. The DBM-A plots were arranged in a 7 x 7 grid pattern with dispensers spaced 26 ft apart (=100/ac). The DBM-B plots were arranged in a 10 x 10 grid with the dispensers spaced 17 ft apart (=200/ac). One sticky trap baited with a Scentry DBM pheromone septum (replaced every 14 d) was positioned near the center of each plot. Once each week, a small, open plastic container with 8 virgin DBM females with one wing clipped was positioned near the center of each block 2-3 h before sunset. Virgin females and also pheromone traps were set up in untreated cabbage nearby, i.e., without pheromone. The moths were retrieved the following morning and dissected to determine mating status.

Results: The DBM-A and -B dispensers were loaded with 400 and 200 mg of total pheromone blend, respectively. The DBM-A dispensers, deployed at 100 sites/ac, were ineffective at preventing mating by DBM. However, the DBM-B formulation, deployed at 200 sites/ac effectively shut down mating of DBM for ca. 40 days. Although 40 days of mating suppression is insufficient to effect season lcng control of DBM in cabbage, the results are encouraging and demonstrate the feasibility of using point sources to effect mating disruption of DBM. If perfected, this method would be much preferred by growers to systems that employ continuous strands of pheromone-dispensing substrate deployed throughout the length and breath of the field.

Plans: Small plot experiments will be conducted to identify pheromone dispensing systems that can be deployed as point sources to control DBM in cabbage.

EFFECTS OF STRIP-CROPPING COLLARDS ON DIAMONDBACK MOTH ABUNDANCE AND ITS PARASITISM BY DIADEGMA INSULARE

E.R. Mitchell, G.Y. Hu[1] and J.S. Okine[1]

Objective: To evaluate the effect of strip-cropped collard greens on diamondback moth and its parasites in cabbage fields.

Methods: Collard greens (*Brassica oleracea var. acephala* L.) were strip-planted between two cabbage fields near Bunnell, Flagler County, Florida in spring 1995. DBM larvae (2nd to 4th instars) on cabbage and collard plants were sampled weekly throughout the growing season. Collected DBM larvae were brought into the laboratory and dissected to determine if they were parasitized, or held for emergence of parasitoids, diamondback moths, or until the larvae died. The larvae were reared in 0.26-liter food cups under laboratory conditions of 21°C, 50 - 60% RH, and 12L:12D. At harvest, 13 consecutive mature cabbage heads >15.2 cm diam were rated for damage using the rating scale developed by Greene et al. (1969) and modified by Leibee et al. (1995). The variation of DBM larval counts and the percentage of parasitism between collard and cabbage plants in both fields were analyzed using ANOVA, and differences between the means were separated with Duncan's multiple range test (DMRT). The raw numbers were transformed by log (n + 1) to meet the assumptions of ANOVA before performance of the analysis.

Results: More larvae of the diamondback moth (DBM), *Plutella xylostella* (L.), were found on collard plants than on cabbage plants in the adjacent fields. Parasitism of DBM larvae collected from the collard plants reached 72% in early May and was higher than for larvae collected from the cabbage plants in adjacent fields. Parasitoids recovered from DBM larvae were mainly *Diadegma insulare* (Cresson). No spread of DBM from collard to the adjacent cabbage was found. The damage to collard plants caused by DBM larvae was greater than to cabbage plants. At harvest, the cabbage heads on the first row next to the collard planting showed less worm damage than did cabbage on the first row on the side of the field away from the collards. However, there was no significant difference in damage ratings of cabbage heads sampled near the middle of the field and damage to heads on rows nearest the collards. The results suggest that collard have potential as a trap crop of DBM in cabbage fields, and that collard can play an important role in maintenance of the natural enemy, *D. insulare*.

Plans: As a result of this work, collard greens have been planted around commercial cabbage fields to protect cabbage from DBM infestation.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

MATING DISRUPTION TRIALS IN CABBAGE: EFFECT OF SHIN-ETSU ROPE FORMULATIONS ON MATING OF DIAMONDBACK MOTH AND CABBAGE LOOPER

E.R. Mitchell, G.Y. Hu[1] and J.S. Okine[1]

Objective: To control diamondback moth (DBM) and cabbage looper (CL) in commercial cabbage with pheromone. All tests were conducted in winter-spring 1995 at Bunnell, FL.

Methods: A total of 88 ac of cabbage was treated with Isomate-DBM pheromone. Sixty-three acres were treated with DBM pheromone only (56 ac in fields a, d, and e with DBM 69025 received Jan. 1995; 12.5 ac in field c with DBM 51201 received Feb. 1994). Field b, 25 ac, was treated with a combination of DBM and CL pheromone(DCM 69024 received Jan. 1995). The cabbage fields were contiguous and ranged in size from 8.8 to 31.6 ac. The cabbage was of mixed age, as each field was planted on a different date ranging from 04 to 20 January. The pheromone was applied within 7 d of planting (range 11 January-02 February). All treatments were applied at rate of 400 yds/ac; attached to stakes 12-14 in. above ground; with each line 8-10 yds from the next. Double strands of rope were applied along rows at field edges, and triple strands of rope were applied from field ends inwards up to ca.15 ft. Treatment efficacy was evaluated by making weekly counts of the number of DBM and CL larvae and pupae per plant. Mating tables with 6-8 sentinel females with one forewing clipped were used to determine the degree that the pheromone reduced mating with wild males. Two mating tables were positioned midway into each field along the length of the row and ca. 1/3 the way in from the sides. Moths were placed in the field 1-2 h before sundown, retrieved the following morning, and dissected to determine mating status. Sentinel females located in nearby cabbage fields treated with conventional pesticides served as controls. Pheromone traps also were set up in each field to determine if the pheromone reduced captures of wild male moths compared to traps in conventionally-sprayed fields. At harvest, 13 consecutive mature cabbage heads >15.24 cm diam at 6+ sites in each field were rated on a scale of 1 to 6 damage categories with 1 showing virtually no damage and 6 showing severe damage to the head and wrapper leaves. Cabbage heads rated ≤ 3 generally are considered marketable under normal market conditions

Results: The DBM and Combo pheromone treatments effectively shut down mating by DBM and capture of males in pheromone traps for >50 days. DBM larval counts did not exceed 0.3 larva/plant, the composite action economic spray threshold (CAST) for cabbage, until the 58th day after the pheromone was applied. Three pesticide sprays were applied in fields d and e; one spray was applied in fields a and b. The conventional spray field was treated 7 times. Adult CL populations were low throughout the season, and CL larval counts on plants were insignificant. The CL-DBM combo treatment reduced mating by CL females and captures of CL males in pheromone traps 100% for 64 days following application. Head damage ratings showed that 92 and 97% of the cabbage in the pheromone- and conventionally-sprayed fields were rated as marketable (difference not statistically significant).

Plans: The 1995 tests will be repeated in winter-spring 1996 using pheromone, *Cotesia plutellae* parasitoids and trap crop plantings.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

TEMPORAL DISTRIBUTION PATTERN OF DIAMONDBACK MOTH AND CABBAGE LOOPER, AND THEIR PARASITOIDS IN CABBAGE FIELDS

E.R. Mitchell, G.Y. Hu[1] and J.S. Okine[1]

Objective: To examine larval population densities and parasitism of the diamondback moth and cabbage looper in cabbage fields during different cropping seasons in Florida.

Methods: Research was conducted near Bunnell, Flagler County, Florida in 1995. Seven commercial cabbage fields were examined during the winter-spring cropping period (January to April 1995) and five during the fall-winter period (October 1995 -February 1996). Larvae of the diamondback moth (DBM) and cabbage looper (CL) were sampled weekly from the time of seedling transplant to harvest. Collected larvae of DBM and CL were brought into the laboratory and dissected under a microscope or held for emerge of parasitoids. Sampling sites were arranged in a grid pattern for all the fields and varied in numbers according to field sizes. Numbers of cabbage plants examined ranged from 65 to 10 per sites based on the crop size.

Results: Densities of DBM larvae were higher in the spring-winter cropping period as compared with the fall-winter cropping period. Densities of CL larvae, on the contrary, were higher in the fall-winter cropping season than the winter-spring season. During the winter-spring cropping season, the earlier the seedlings were transplanted, the less DBM larval infestation was found on the cabbage plants, and the less pesticide was applied. During the fall-winter cropping season, the earlier the seedlings were transplanted, the more severe CL infestation was observed on the plants, and the more pesticide was applied. Parasitism of DBM and CL were higher in the winter-spring cropping season as compared with the fall-winter season when parasitism remained almost zero. Parasitoids recovered from DBM larvae were *Diadegma insulare* (Cresson) and *Conura side* (Walker), and from CL larvae were *C. marginiventris* (Cresson) and *Copidosoma* sp. Our results suggest that control strategies for cabbage pests may vary from season to season of the year in this cropping area.

Plans: Continuous examinations have been planned for the fields in the same cropping area in 1996.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

LARGE-SCALE FIELD TEST OF SHIN-ETSU PHEROMONE MATING DISRUPTANT FORMULATION FOR CONTROL OF BOLLWORM AND ARMYWORM COMPLEX IN COTTON

E.R. Mitchell, J.S. Okine[1] and G.Y. Hu[1]

Objective: To control corn earworm (CEW), tobacco budworm (TBW), beet armyworm (BAW), and fall armyworm (FAW) in cotton by disrupting mating using excessive quantities of synthetic sex pheromone evaporated from Shin-Etsu 'ropes'.

Methods: A total of 200 ac of cotton was treated with pheromone. The Trenton field (115 ac) was treated with BAW (BAW 70302) and FAW (FAW 75024) pheromone; the field at Newberry (85 ac) was treated with these and a pheromone blend for CEW and TBW (CTW 75026). All pheromones were received 12 June 1995. Each rope was 8-in. long and contained ca. 160 mg total pheromone blend. The 8-in. long dispensers were tied 8-10 in. above the soil, and spaced ca. 17.3 ft apart throughout ea field. The BAW and FAW ropes were tied 2/site (=400/ac) and the CTW ropes were tied 4/site (=800/ac). The ropes were applied when the plants were in the 8-10 leaf stage (Trenton, 31 July; Newberry, 08 Aug.). Another cotton field of 240 ac located 13 mi north of the Newberry site and 21 mi northeast of Trenton served as the control. There was no other cotton in the general area of the test sites. Treatment efficacy was measured by shutdown of male moth captures in wire cone traps (CEW and TBW) or bucket traps (BAW and FAW) baited with pheromone (2 traps ea/field); reduction in matings by sentinel females (6-8) positioned on mating tables (2 ea/field); and weekly counts of eggs and larvae in ea field. Pheromone traps and mating tables were positioned 1/3 of the way in from the sides of the field. Mating tables were located diagonally across the field from the pheromone traps. This allowed continuous operation of pheromone traps, even on nights that sentinel females were set out on mating tables. At each location, traps or mating tables were positioned ca. 50 ft apart to minimize any possible pheromonal interference between species.

Results: The rope formulation for BAW shut down mating for only ca. 6 wk compared to season-long suppression of mating by BAW in large scale field trials in cotton in 1994. Explanation: We received the wrong pheromone formulation. The dispensers received in 1995 were clear rather than the brown-type used in 1994. The FAW pheromone was ineffective at shutting down mating between sentinel females and wild males. In small plots trials in 1991, a clear dispenser formulation for FAW was extremely effective at shutting down mating for >2.5 months. The 1995 dispensers were clear upon receipt, but turned yellow within a few days after being placed in the field. According to Shin-Etsu, the yellow color was due to vitamin E which was added to the formulation to protect the pheromone from degradation. The CTW formulation effectively shut down captures of CEW males in traps and mating by sentinel females for 30 days; the treatment was more effective against TBW lasting ca. 60 days. Natural control was so pervasive in all fields that it was impossible to obtain quantitative data on the effectiveness of the pheromone-treatments in reducing worm infestations and plant damage relative to the control. None of the fields required more than 3 sprays with pesticides.

Plans: If formulated pheromone materials are available, mating disruption trials for BAW, CEW, and TBW will be repeated.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

LOW TEMPERATURE EFFECT ON VIABILITY OF *DIADEGMA INSULARE* (HYMENOPTERA: ICHNEUMONIDAE) PUPAE AND EFFECT OF *D. INSULARE* PARASITISM ON FEEDING RATE OF DIAMONDBACK MOTH LARVAE

J.S. Okine[1], E.R. Mitchell and G.Y. Hu[1]

Objective: To determine the viability of *D. insulare* pupae stored at 4°C for varying lengths of time, and also to measure foliage consumption rate of parasitized and unparasitized diamondback larvae.

Methods: Effect of low temperature on survival: Field collected *Diadegma insulare* pupae were stored in a Nor-Lake™ environmental chamber at 4°C in mid-May 1995. A sample from the collection was used as control and not subjected to cold conditions. Each week for 7 consecutive weeks after collection, varying numbers of pupae were collected and set up in the laboratory under normal ambient conditions (25±2°C and 70-80% RH). Adult emergence was followed for 25 days for each set of pupae taken out. Emerged adults were aspirated and sexed. The control group was also followed similarly. After 25 days unemerged pupae were dissected. Consumption of collard by parasitized and unparasitized DBM larvae: Two groups of larvae, made up of four replicates each were set up. Each replicate had 20 second instar DBM larvae. The larvae were held in 250 ml Dixie™ cups with ventilated covers and fed fresh collard (*Brassica oleracea* var. acephala) leaves daily. One group was exposed to 5, 4-day old mated *D. insulare* females per replicate. Stinging was allowed for 24 hours. The other group was untreated and used as control. Foliage consumption was assessed by measuring feeding areas with a plastic mm^2 grid. Leaf measurements were made for 5 days. The experiment was carried out under ambient laboratory conditions.

Results: Results of the *D. insulare* pupal viability test indicated that adult emergence decreased linearly with increasing storage time at 4°C. Viability was reduced >50% after 21 days of storage. Foliage consumption rate was significantly different between parasitized and unparasitized DBM larvae. These results show clearly that after DBM larvae are parasitized by *D. insulare,* they no longer pose a threat to growers' cabbage crops. If ways can be found to increase the numbers of *D. insulare* in cabbage, either through releases or habitat manipulation, this parasitoid could become an important biocontrol control agent for DBM in Florida and elsewhere.

Plans: This work has been completed.

[1] Post-doctoral associates, Department of Entomology and Nematology, University of Florida.

HOBO-MEDIATED TRANSFORMATION OF *D. VIRILIS*

S.P. Gomez[1] and A.M. Handler

Objective: To test the ability of the *D. melanogaster hobo* gene-transfer vector to mediate germline transformation in *D. virilis* and to test the ability of the *D. melanogaster white+* marker gene to complement the *D. virilis white* mutation.

Methods: In separate experiments we tested the heat-shock regulated *hobo* helper (pHSH2) and unmodified *hobo* (pHFL1) helper to mediate gene transfer of the H[*w+hawN*] vector carrying the *D. melanogaster* mini-*white+* cassette inserted within a partially deleted *hobo* element. In the pHFL1 helper experiment 600 *D. virilis white*$^{50\text{-}112}$ host embryos were injected and 265 surviving G0 adults were intermated in 73 lines. G1 offspring were analyzed for red eye coloration and outcrossed to *white* mutant flies, with subsequent inbreeding of red-eye (*white+*) offspring. Chromosomal linkage was determined by outcrosses, with southern blot and *in situ* hybridization used to molecularly verify chromosomal insertion and determine specific genomic insertion sites.

Results: Precise *hobo* excision observed in transient assays with *D. virilis*, comparable to *D. melanogaster*, suggested that *hobo*-mediated transformation might also occur at comparable levels in *D. virilis*. Since significant homology exists between the *D. melanogaster white+* gene and those cloned from other insects we attempted *hobo* transformation of a *D. virilis white* strain using a vector carrying the *D. melanogaster* mini-*white+* marker. From one line (Dv[haw]-73) five G1 offspring were observed having a strongly expressed red-eye phenotype. Independent lines were created for each G1, with one lost due to a failure to reproduce. Subsequent southern and salivary gland chromosome *in situ* hybridizations indicated that all G1 transformants occurred from a single event, with vector insertion occurring at position 19A/B on the X-chromosome. We estimate a transformation frequency of approximately 0.5 to 1% (based on typical G0 fertility of 70%). Salient information from this experiment is that i) the *hobo*transposon can mediate gene transfer in a distantly related drosophilid (divergence occurring more than 40M yr ago), ii) the *D. melanogaster white+* gene can almost fully complement the *D. virilis white* mutation, and iii) that the unmodified *hobo* promoter is functional in *D. virilis*. Of considerable interest in this study was the recovery of several mutant phenotypes in G0 and G1 offspring, suggesting that *hobo* may have mobilized an endogenous *hobo*-related element. *hobo*-mediated transformation of *D. virilis* suggests that more distantly related species to *D. melanogaster* may also be transformed with this vector, though probably at lower frequencies.

Plans: Transformation experiments with the *hobo* vector will be performed in tephritid fruit fly species. The ability of *D. virilis* to cross-mobilize *hobo* in transient assays (possibly due to a *hobo*-related element) suggests that *hobo* transformed strains may not be stable. Stability of *D. virilis* transformants will be monitored in mass-reared strains by eye color inspections and chromosomal *in situ* hybridization.

[1] Post-doctoral associate, Department of Entomology and Nematology, University of Florida.

ISOLATION OF A *HOBO*-RELATED ELEMENT FROM *BACTROCERA DORSALIS*

A.M. Handler and S.P. Gomez[1]

Objective: To isolate a tephritid transposable element for development into a vector for efficient and stable germline transformation.

Methods: A *hobo*-related element subfragment (Bd-HRE) was isolated by polymerase chain reaction from *B. dorsalis* using primers derived from homologous amino acid sequences in the *hobo* and Ac transposable elements. To isolate a complete Bd-HRE transposon, the PCR product was used to screen a *B. dorsalis* genomic library created from LambdaGEM11 (Promega) and wild Kahuku strain genomic DNA. Several positive plaques were selected, subcloned and sequenced. Additional element sequence was obtained from inverted PCR reactions.

Results: An apparently complete, but probably nonfunctional 3,120 bp element (which we tentatively name *hopper*), bounded by 19 bp inverted terminal repeats, was sequenced from two independent clones and a separate element was partially sequenced from inverted PCR products. The cloned element has two long overlapping open-reading frames (ORF). The Bd-HRE PCR sequence encodes a complete ORF which overlaps the discontinuous region between the genomic ORFs. Two base changes present in the PCR sequence result in a single consensus ORF of approximately 1.5 kb in the genomic clone. The insertion site of one element has an 8 bp direct repeat sequence adjacent to both termini and the inverted termini contain a 2A; 5G motif consistent with other members of the *hobo*, *Ac, Tam3 (hAT)* family of transposable elements. Several direct repeats also occur within the element. A comparison of *hopper* to all amino acid sequences in the Gen Bank using BLAST shows greatest similarity, although a limited relationship, to the *Ac, hobo,* and *Hermes hAT* elements. Though, apparently, a member of the *hAT* family, the *hopper* element is the most divergent of the insect elements.

Plans: We are presently sequencing additional genomic clones to resolved the ORF discrepancy. Putative autonomous elements will be tested for functionality in excision/transposition assays previous to use in transformation experiments.

[1] Post-doctoral associate, Department of Entomology and Nematology, University of Florida.

ASSOCIATIVE LEARNING OF HOST ODOR BY CABBAGE LOOPER MOTHS

P.J. Landolt and O.H. Molina

Objective: To determine if attraction of mated female cabbage looper moths to host plants increases following oviposition experience on such host plants. Such an increase would be evidence of associative learning of host odor and could explain how orientation to host odors by a generalist herbivore would facilitate the finding and selecting of quality host plants.

Methods: A flight tunnel bioassay was used to evaluate attraction responses of mated female cabbage loopers to host plants. Cabbage loopers were obtained as pupae from the laboratory colony and were held in screened cages, with water and sugar solution, in a greenhouse until adult emergence. Females were placed overnight in cages with males when 2 to 3 days old. Ninety to 95% of such females were mated over the course of the study. On the night following mating, one cohort of females was placed in a cage with a bouquet of foliage in a flask of water, as oviposition experience. The foliage was removed the following morning and 10 of the moths (experienced) were tested in a flight tunnel for attraction to host plant foliage the following night, when 4 to 5 days old. A second cohort of moths was held in similar cages, but without any host plant foliage. Ten of these moths (naive) were also tested the following night for attraction to host plant foliage. This experiment was conducted on 5 different days using cotton foliage both for oviposition experience and in the flight tunnel bioassay. The same experimental design was then used to evaluate experienced and naive moth responses to celery foliage, with celery used both for oviposition experience and in the flight tunnel bioassay. Cotton (Germaine 510) and celery (Utah tall) were grown from seed in pots in a greenhouse. Mean percentage response data from the flight tunnel bioassay were compared using Student's t-test.

Results: Female cabbage looper moths that had been caged with cotton foliage were significantly more likely the following night to exhibit upwind oriented flights (attraction) to contact cotton foliage (41.1 $\pm$ 2.5%) than were inexperienced moths (5.0 $\pm$ 3.1%) ($t=9.2$, $df=8$, $p=1.6 \times 10^{-5}$). . Similarly, female cabbage looper moths that had been caged with celery foliage were significantly more likely the following night to exhibit attraction to contact celery foliage (40.6 $\pm$ 4.1%) than inexperienced moths (2.0 $\pm$ 2.0%) ($t=6.6$, $df=8$, $p=1.2 \times 10^{-3}$). These results indicate that cabbage looper moths may learn host odor upon contact with host foliage, providing a specific host cue useful in host finding.

Plans: This study has been completed.

ATTRACTION OF TOBACCO BUDWORM MOTHS WITH A FERMENTED SWEET BAIT

P.J. Landolt and E.R. Mitchell

Objective: To determine if tobacco budworm moths, *Heliothis virescens*, are attracted to and can be trapped with fermented sweet baits. A small number of tobacco budworm moths were captured in traps in a previous study of grass looper attraction to sweet baits.

Methods: Jaggery, a palm sugar extract, was used as the sweet bait. For each assay, an aqueous solution of jaggery was placed on a 200 ml of tap water and placed in a 500 ml glass jar and then placed on a laboratory shelf until tested. A batch of jaggery was tested for attractiveness to male tobacco budworm moths as bait in a glass McPhail trap commonly used for capturing tephritid fruit flies. A similar trap containing 200 ml of tap water was used as a control. Traps were hung from the ceiling of a cylindrical screened field cage (2.7 x 2.3 m) and were one m apart and 0.3 m from the cage ceiling. For each assay, a control trap and a baited trap were placed in a field cage in which moths were released. Numbers of moths captured were counted the following day. Two field cages were used and treatment positions in each cage were alternated. Tobacco budworms were obtained from the laboratory colony as pupae and were held in screened cages in a greenhouse on a natural light cycle until 3 to 5 days old when they were released into a field cage. Moths were provided water on cotton within each emergence cage and a water bottle on top of each emergence cage, but were not provided food. Batches of 20 to 30 moths were released per cage, but not for all replicates. If abundant live moths remained in a field cage following an assay, another assay was conducted without releasing more moths. This assay was replicated 18 times during February and March. Mean trap catch data was analyzed by Student's t-test.

Results: While no male tobacco budworm moths were captured in control traps baited with tap water, a total of 74 of 260 moths released were recaptured in traps baited with jaggery. Mean trap catches were 4.1 ± 1.6 for jaggery baited traps. These results were significantly different by Student's t-test ($T=2.5$, $df=34$, $p=0.05$).

Plans: Studies are underway to develop a flight tunnel assay to be used in efforts to optimize such sweet baits and isolate attractive volatiles. Similar experiments will be conducted to determine if adult *Helicoverpa zea*, *Spodoptera exigua* and *Spodoptera frugiperda* are attracted to aged sweet baits.

SYNERGISM OF A CABBAGE LOOPER, *TRICHOPLUSIA NI* (HüBNER), SEX PHEROMONE SPECIALIST NEURON BY THREE HOST-PLANT COMPOUNDS

M.S. Mayer and J.C. Dickens[1]

Objective: To determine whether the Z7-12:Ac specialist of the cabbage looper, *Trichoplusia ni* (Hübner), is synergized by some volatile host plant emissions.

Methods: Responses of the HS(a) Z7-12Ac specialist receptor neuron of the cabbage looper moth were recorded extracellularly with a sharpened tungsten electrode. Because of the relatively high volatility of hexyl acetate and hexanol, the host plant stimuli were dispensed from rubber septa. The emission rate of the host plant compounds was estimated from first principles established by McDonough (ACS Symposium Series No. 449, p. 106, 1991). The action potentials were digitized, recorded and counted by means of a computer. Behavioral responses to mixtures of host plant compounds and Z7-12:Ac were assayed in a wind tunnel. The wind tunnel assays demonstrated whether or not the mixture synergized upwind flight and copulation. From estimates of evaporation rates, the airborne concentration of the host odor stimuli requisite to elicit synergism was compared with what may be found immediately downwind from a host plant.

Results: The response of the HS(a) specialist neurons to Z7-12:Ac was synergized by the volatile host plant compounds, hexyl acetate and hexanol, and the less volatile compound, linalool. The wind tunnel assays confirmed that both upwind flight and copulation were synergized by the host plant compounds. The question of whether or not the host plant compounds can synergize the response of the HS(a) specialist receptor neuron in nature was examined by comparison of the emission rate of the host plant compounds and their dilution by air. The concentrations of these compounds in the air was sufficient to synergize the HS(a) specialist response to Z7-12:Ac, and exceeded expected airborne concentrations in the field.

Plans: There are no plans for further work on this project.

[1]USDA-ARS, Beltsville, Maryland.

FIELD TESTING OF A PORTABLE PHEROMONE MONITORING DEVICE

M.S. Mayer and E.R. Mitchell

Objective: To develop a portable device that relies on the electroantennogram (EAG) to monitor and measure pheromone levels in field environments.

Methods: A portable, commercially-available high input impedance biological preamplifier that has been incorporated into a proprietary mounting supporting a live insect was used to obtain EAG's in pheromone treated field plots. For analysis of EAG potentials, voltages from the EAG were recorded by a datalogger every 6.25 ms and downloaded into an ASCII file. The variance of the voltage shifts were analyzed by a SAS program. Ten recordings were made at three heights in the center of a 40 acre pheromone-treated cotton field and in the center of an untreated field for a control variance estimate. Another control measure was made in treated fields by closing the lid of the EAG chamber which seals the antenna from ambient air.

Results: It is not likely that the absolute airborne pheromone concentration can be measured with the device (Rumbo et al. J. Insect Physiol. 41:465, 1995). However, relative estimates of the presence or absence of pheromone may be obtained for correlation with trapping results and mating assays. Samples of recordings demonstrate that the EAG excursions from the baseline are greater in the open air in a treated field than they are in clean air. There also was no statistical difference between the variances obtained in the blank recordings and EAG's obtained in an untreated field. Furthermore, there appears to be a time between the 6th and 13th of Sept. when the EAG variances in the treated field were no greater than the EAG variances in the untreated field. At about the same time, mating began to increase in the mating assays. Thus, we associate the increased EAG variances observed earlier with detection of pheromone and consider that the use of EAG measurements may be an alternate method to assess the potency of pheromone in treated fields.

Plans: The EAG apparatus will be tested in pheromone treated cabbage fields and cotton fields during 1996. The results will be correlated with the results of field trapping and mating assays.

THE RELATIVE ABUNDANCE OVER TIME OF TWO BRACONID PARASITOIDS OF THE CARIBBEAN FRUIT FLY (*ANASTREPHA SUSPENSA* (LOEW)) (DIPTERA: TEPHRITIDAE)

J. Sivinski, M. Aluja[1], T. Holler[2] and A. Eitam[3]

Objectives: To determine temporal changes in the distribution of the 2 major parasitoids of the Caribbean fruit fly in an area where both are abundant. Information on the conditions that favor the different species will suggest which is the appropriate candidate for augmented releases at particular places and times.

Methods: Fruit where collected in LaBelle, Florida, year-round, for 2 years, from 5 species of host trees. The larvae that emerged were allowed to pupate and held in moist vermiculite. After a 1 month period for emergence the adult insects were identified.

Results: Two species of Braconidae, *Diachasmimorpha longicaudata* (Ashmead) (=Dl) and *Doryctobracon areolatus* (Szepligeti) (=Da), commonly attack the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in central Florida. In this area of co-occurrence, there were temporal changes in the relative abundance of the parasitoids. In a focal study tree, Dl became more abundant, actually and relative to Da, as the fruiting period progressed. Three hypotheses were examined, using data from the focal and other local host trees, for their ability to account for this trend. 1) Da may be superior to Dl in finding host patches, but is inferior at exploiting hosts: "Counter-balanced competition" fails to be supported in one of 5 fly-host plant species, the small fruited citrus, calamundin. In calamundin Dl becomes less abundant relative to Da through the autumn fruiting period. 2) Within-tree changes in fruit density, size or infestation levels over time can account for the ratio of Dl to Da: There is no evidence to implicate changes in hosts over time to relative rates of parasitism. 3) Seasonal changes in the environment favor one species over the other: In general, Dl becomes more abundant during the spring and declines in the fall. This seasonal pattern is closely correlated to factors such as mean high and low temperatures. Climate, or some unknown biotic correlate(s) of climate, best explains the fluctuations in relative abundance.

Plans: Dl is used in augmented releases to protect fly-free citrus producing zones. Information on the environmental tolerances of this and alternative parasitoids may allow future releases to be tailored to local conditions.

[1]Instituto de Ecologia, A. C., 91000 Xalapa, Veracruz, Mexico
[2]USDA-APHIS, c/o Florida Division of Plant Industry, Gainesville, Florida
[3]Department of Entomology and Nematology, University of Florida

THE SPATIAL AND TEMPORAL DISTRIBUTIONS OF PARASITOIDS OF MEXICAN *ANASTREPHA* SPECIES (DIPTERA: TEPHRITIDAE) WITHIN THE CANOPIES OF FRUIT TREES

J.M. Sivinski, M. Aluja[1] and M. Lopez[1]

Objectives: Information on the foraging behaviors and distributions of parasitoids within host trees may predict which natural enemy introductions will fill "gaps" in the present fauna attacking the Caribbean fruit fly. That is, are there microhabitats in tree canopies where particular parasitoids are most effective? If so, will further establishments of specialized parasitoids allow fly larvae to be attacked that are presently escaping parasitism?

Methods: In Veracruz State, Mexico the temporal and spatial distributions of 5 species of parasitic Hymenoptera attacking 5 species of *Anastrepha* in 7 species of fruit tree canopies were examined. The parasitoids are abbreviated as follows: *Dorystobracon areolatus* (Braconidae)=Da; *D. crawfordi*=Dc; *Diachasmimorpha longicaudata* (Braconidae)=Dl; *Utetes anastrephae* (Braconidae)=Ua; *Aganaspis pelleranoi* (Eucoilidae)=Ap.

Results: Parasitism by Da, Dl and Ua was higher in 3 of 4 significant cases in the lower portions of the canopies. Ua was more abundant in the interior of canopies (2 cases), while Da was more common in the margins (1 case). In 6 of 7 instances representing all species the mean size of fruits containing parasitoids was smaller than that of infested fruits without parasitoids. Ua attacked larvae in a narrow range of smaller host species relative to other parasitoids. The efficiency (proportion of larvae attacked in a fruit) of Dl compared to that of other parasitoids increased with fruit size. Dl may be better able to locate and/or attack hosts in larger fruits. In all of 17 instances there were on average more host pupae in fruits containing parasitoids of all species than in fruits without parasitoids. In all of 18 significant instances the larval density (pupae/gr of fruit) was higher in fruits that contained parasitoids of any species than in fruits that did not. Parasitism by Da, Dc, Dl and Ua often changed over time during the fruiting period, but was as likely to decrease as increase. In Da there was a pattern of decreasing parasitism during the fruiting periods of individual trees as the season changed from rainy to dry. There were only a few instances of significant relationships between parasitism and local differences in the canopy in terms of fruit numbers, host numbers and host density. In 2 instances there were significant negative relationships between parasitism caused by the commonly co-occurring Da and Ua. In 2 other cases parasitism by Dc and Dl was positively correlated. Dl is a recent introduction to Mexico and the positive relationships may indicate a niche overlap not present between the 2 endemic species, Da and Ua. Fewer than expected fruits containing Da and Ua is further evidence of niche differences. This pattern did not occur in fruits containing Dc and Dl.

Plans: Information on the distribution of parasitoids at levels ranging from within canopies to across regions may guide biological control efforts, allowing the match of candidate species to locations. These data will be used in combination with information on the distributions of parasitoids already present in Florida to guide future introductions of natural enemies.

[1]Instituto de Ecologia, A. C., 91000 Xalapa, Veracruz, Mexico

COMBINED RELEASES OF PARASITOIDS AND STERILE MALES TO CONTROL MEDITERRANEAN FRUIT FLIES

J.M. Sivinski and T.C. Holler[1]

Objective: United States agriculture is protected from the Mediterranean fruit fly by a fly-free barrier along the Mexican/Guatemalan barrier. This barrier has been maintained for over 15 years through the use of sterile-male releases and Malathion-bait sprays. However, the use of insecticides is soon to be further limited and no techniques for suppressing fly populations are required. The possibility of combining parasitoids and sterile male releases is being examined through a cooperative USDA-ARS, USDA-APHIS, and MOSCAMED program.

Methods: Previous experiments had shown the braconid *Diachasmimorpha tryoni* to be best suited to region. In 1996 combined releases are being made over a 12 square km block. Controls consist of similar blocks which are untreated or in which flies alone are released.

Results: The data for 1996 are not yet completely collected. At the time of writing, fly populations are at seasonal low points throughout the study area.

Plans: Larger scale releases will be made In 1996-1997. Rearing technologies for mass rearing parasitoids are simultaneously being developed. New species of parasitoids from both the Old and New Worlds will be examined for their suitability either in classical or augmented releases.

[1]USDA-APHIS, c/o Division of Plant Industry, Gainesville, FL

THREE SPECIES OF PUPAL PARASITOIDS (HYMENOPTERA) CONSIDERED AS CANDIDATES FOR AUGMENTED RELEASES AGAINST TEPHRITID FRUIT FLIES (DIPTERA)

J.M. Sivinski, K. Vulinec[1] and M. Aluja[2]

Objective: Because tephritid pupae are sometimes more vulnerable than larvae, pupal parasitoids are attractive candidates for augmented releases to control pest fruit flies. However, a number of such species have broad host ranges and act as hyperparasitoids of braconid primary parasitoids. This work is part of a project to identify pupal parasitoids that do not have these drawbacks and will be suitable for augmented releases.

Methods: Three pupal parasitoids were examined for: 1) host range (i.e. ability to develop in distantly related hosts; the house fly, *Musca domestica*, and the Caribbean fruit fly, *Anastrepha suspensa*); 2) whether host range is modifiable (i.e. would rearing in a particular host change adult response in an olfactometer to that host) and 3) propensity to hyperparasitize a braconid parasitoid of Tephritidae, *Diachasmimorpha longicaudata*.

Results: The chalcid *Dirhinus himalayanus* developed in both hosts, though it was more responsive to the pupae of house flies. Rearing in Caribbean fruit flies did not significantly affect this response. It avoided hyperparasitism to some extent (mortality of the primary parasitoid was ~ 2/3 that of the host fly). The pteromalid *Spalangia gemina* also preferred the pupae of house flies, but the response to house fly pupae was significantly less in insects reared on Caribbean fruit flies. *S. gemina* acted as a hyperparasitoid and did not appear to discriminate between parasitized and unparasitized pupae of Caribbean fruit flies. The diapriid *Coptera* sp. did not develop in the pupae of house flies and was responsive only to the pupae of Caribbean fruit flies. It did not act as a hyperparasitoid.

Plans: Mass-rearing schemes using both larval parasitoids and pupal parasitoids that avoid hyperparasitizing are may be possible using *Coptera* sp or some similar insect. This could increase the efficiency of rearing operations and make releases a better means of pest population suppression.

[1]Dept. of Entomology and Nematology, University of Florida
[2]Instituto de Ecologia, A. C., Apartado Postal 63, Xalapa, Veracruz, Mexico

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DEVELOPMENT OF ARTIFICIAL MEDIA AND CULTURE SYSTEMS FOR SELECTED INSECT PARASITOIDS AND PREDATORS

P. Greany, J. Carpenter[1], D. Weaver, R. Weseloh[2],
T. Coudron[3], S. Sing[4] and T. Arbogast

Objective: To develop a low-cost artificial medium and related presentation system for the mass rearing of economically important insect parasitoids and predators.

Methods: A simple artificial medium that does not incorporate any insect components has been developed using readily-available materials. Methods have been developed to encapsulate and sterilize the medium. Because the ARS Patent Committee is pursuing a patent on the methods and materials, additional details cannot be provided at this time.

Results: Several species of insect predators and parasitoids have been successfully reared on the medium in question, including: *Diapetimorpha introita, Cryptus albitarsus, Calosoma sycophanta, Coleomegilla maculata, Xylocoris flavipes, Lyctocoris campestris, Podisus maculiventris, Perillus bioculatus,* and *Geocoris punctipes*. These in turn attack a wide array of insect pests.

Plans: CRADAs have been established with two private firms (Analytical Research Systems, Inc., and Predation, Inc.), to develop procedures and equipment to enable scaled-up production and encapsulation of the medium. An SBIR has been awarded to Analytical Research Systems to develop the encapsulation procedures. A major emphasis will be placed upon production of large numbers of *Podisus maculiventris* for release against the diamondback moth and the cabbage looper in cabbage.

[1]USDA ARS Insect Biology & Population Management Research Laboratory, Tifton, GA
[2]Connecticut Agricultural Research Station, New Haven, CT
[3]USDA ARS Biological Control of Insects Research Laboratory, Columbia, MO
[4]Dept. Natural Resource Sciences, McGill University, Montreal, Canada

PILOT TEST: USE OF GIBBERELLIC ACID FOR FRUIT FLY CONTROL IN CITRUS IN FLORIDA AND MEXICO: FINAL REPORT

P. Greany, R. McDonald[1] and M. Aluja[2]

Objective: To use gibberellic acid to retard citrus fruit senescence and thereby delay the onset of fruit fly susceptibility.

Methods: Gibberellic acid (GA) treatments were applied to citrus trees in commercial groves using locally-appropriate spray equipment (speed sprayers in Florida, mechanically-powered hand sprayers in Mexico). Application was made prior to fruit colorbreak (in August in Florida, earlier in Mexico). Treatments were applied using 10-20 g of GA per acre, in combination with the surfactant Silwet L-77 at 0.05% (OSi, Inc.). Periodic samples of treated vs. untreated fruit were made to assess leaf drop, fruit peel color and peel firmness. Bioassays of fruit susceptibility to fruit flies were performed by exposing treated and untreated fruit together in field cages inoculated with *Anastrepha suspensa* (in Florida) or *A. ludens* (in Mexico). In addition, infestation by wild flies was determined at each location by holding fruit from treated vs. untreated trees for fly development. Tests in Florida involved only 'Marsh' var. white grapefruit; in Mexico, tests were conducted with both grapefruit and oranges.

Results: Application of 10-20 g/acre of GA in 250 gallons of spray solution had a demonstrable, dose-dependent effect upon initial leaf drop and upon fruit color and resistance to puncture both in Florida and in Mexico. GA-treated fruit proved to be less susceptible than untreated fruit to fruit fly attack in field cage tests in Florida. No infestation occurred in either GA-treated or untreated fruit from a grove exposed to a wild fly population. Results from Mexico suggest that GA treatment alone does not provide assured protection of grapefruit from the Mexican fruit fly, but along with use of malathion bait sprays, sufficient protection may be obtained. Mexican oranges rarely are attacked by A. ludens, except very late in the season. GA treatments reduced spontaneous abscission of Florida and Mexican grapefruit and oranges, enabling extended on-tree storage of the fruit. The value of fruit kept on the tree through March as a result of the GA treatment was about twice as great as the cost of the GA treatment.

Final Conclusions: GA treatment of grapefruit provided increased protection against the Caribbean fruit fly in Florida grapefruit. This approach may provide a viable alternative to use of malathion bait sprays for use in the Fly Free Protocol. In Mexico, use of GA will be most beneficial in extending the harvest season for grapefruit and oranges.

[1]U.S. Horticultural Research Laboratory, Orlando, FL
[2]Instituto de Ecologia, A.C., Xalapa, Veracruz, Mexico

ECONOMICAL REARING OF LARVAL-PUPAL PARASITOID *ARCHYTAS MARMORATUS* (DIPTERA, TACHINIDAE) ON ARTIFICIAL MEDIA: POTENTIAL OF LOW-COST MEDIA OF NON-INSECT ORIGIN

S. M. Ferkovich and C. R. Dillard

Objective: To examine the potential of using two artificial diets and support surfaces for rearing *Archytas marmoratus*, a parasitoid of Noctuid species.

Methods: An artificial medium, which was developed by Dr. P. Greany and is currently being tested on several entomophages and an agar diet provided by G. Rojas (Subtropical Agri Res Lab. ARS, USDA) was evaluated on three support systems. The support systems, powdered and granular polymerized acrylamide and cotton balls were used so that the larvae could place their rear tracheal spiracles above the liquid surface. Diet was either autoclaved or gamma irradiated for sterilization. First instar larvae were dissected from the reproductive tracts of gravid females under sterile conditions and placed into media in culture plates which were then sealed and held at 27°C. In separate experiments, 20-hydroxyecdysone (10^8 to 10^6 M) was added to stimulate molting to the second instar.

Results: None of the treatments were effective in supporting growth of the parasitoid beyond the 7 th day and none molted into the second instar. All larvae were dead by day 7. Larvae on the autoclaved diets were more active during the first two days than were those on irradiated diet.

Plans: No further work on this project is planned at this time.

ECONOMICAL REARING OF *MICROPLITIS CROCEIPES* ON ATYPICAL HOSTS: ROLE OF PARASITOID POLYDNAVIRUS IN SUCCESSFUL PARASITIZATION OF UNNATURAL HOSTS

S. M. Ferkovich, P. Gupta and C. R. Dillard

Objective: To compare the effects of calyx fluid (polydnavirus) and venom from the reproductive tract of *M. croceipes* in suppressing the immune response and growth and development of the natural host *H. zea*, and two atypical hosts, *G. mellonella* and *S.* exigua.

Methods: Calyx fluid and virus were collected from reproductive tracts of ♀ *Microplitis croceipes* under sterile conditions and injected into larvae of the natural host, *H. zea*, and atypical hosts *G. mellonella* and *S.* exigua. The larvae were injected with 0, 0.05, 0.1 and 0.2 ♀ equivalents of calyx fluid, venom, and caly x fluid plus venom in combination. Calyx fluid was also purified using Centrex membrane filters, and stored at -80° C. Total hemocyte counts were made 48 hrs after injection with 0.2 ♀ equiv. of calyx fluid plus venom. Injected larvae were weighed daily until pupation and adults were weighed on the day of emergence.

Results: Earlier, we successfully reared *M. croceipes* on *G. mellonella*, but not on *S. exigua*, both of which are atypical hosts this suggested that the polydnavirus was partially effective in suppressing the immune system *of G. mellonella*, but not effective in *S. exigua*, since 100 % of the oviposited eggs were encapsulated in parasitized *S. exigua* larvae. In this study, we hypothesized that the polydnavirus associated with *M. croceipes* affects the development of the natural host, *H. zea*, is minimally effective in *G. mellonella*, and ineffective in *S. exigua*. Our results clearly show that injections of the calyx fluid and venom even in the absence of parasitoid, reduced growth and development in *G. mellonella* and these effects were similar to the effects of parasitized larvae. In the natural host *H. zea*, venom had no effect on growth although calyx fluid did have an effect, but the effect was less than calyx fluid plus venom. *M. croceipes* had the least immuno-defensive response to *S. exigua;* there was no significant effect on larval weight gain although the developmental period from injection to pupation was increased and adult weights were reduced significantly with injections of calyx fluid plus venom. In *G. mellonell*a, calyx fluid, venom and calyx fluid plus venom reduced larval growth, % pupation, % emergence, and adult size and prolonged the developmental period from injection to pupation. Total hemocyte counts were significantly higher in *H. zea* and *S. exigua* after virus plus venom injections, but cell numbers remain unaffected in *G. mellonella*. In *G. mellonella*, the unaffected cell numbers after polydnavirus injections further supports that polydnavirus has a weak effect on its immune system, and thus facilitates development of *M. croceipes* in this species. In conclusion, these data indicate that calyx fluid and venom from *M. croceipes* can differentially interfere with the growth and development of atypical hosts *G. mellonella* and *S. exigua* in addition to its natural host, *H. zea*. In preparation for future viral expression studies, we found that both calyx fluid and purified polydnavirus lost activity during storage at -80° C.

Plans: Future investigations will focus on the expression and distribution of viral DNA in various tissues of atypical hosts compared with the natural host.

CHEMISTRY

CRIS - 6615-22000-012-00D -- Chemistry and Biochemistry of Insect Behavior, Physiology, and Ecology

INDUCTION OF OVIPOSITION IN VIRGIN FEMALES OF *HELICOVERPA ZEA*.

R.L. Abernathy and P.E.A. Teal.

Objectives: To determine the endogenous mechanisms responsible for stimulation of oviposition in virgin females of Helicoverpa zea.

Methods: The bursa copulatrix, which, in senescing virgin females, contains pheromone biosynthesis suppression factor, was dissected from newly eclosed and 5-day old females. The corpus bursa (CB) was isolated from the cervix bursa and placed in sterile Graces medium containing 0.005 mg/ml gentamicin and a few crystals of phenylthiurea. The CB was then implanted into newly eclosed females. Prior to implantation the lateral side of the abdomen was swabbed with 70% ETOH followed by Graces medium and a lateral incision was made between the 3rd - 4th abdominal sclerites. The CB was placed into the abdomen and the wound was closed. Sham operated insects were treated in a similar fashion but no CB was implanted. After implantation insects were placed individually into cages and maintained in a growth chamber at 25°C, 65% RH and under a 14:10 L:D photoperiod. Each female was provided with a cotton dental wick soaked in 5% sucrose solution. The total number of eggs laid were counted every 24 h for 3 days.

Results: Sham operated females deposited an average of 19 (± 7.9, SEM, N = 15) eggs over the three days of study. Similarly, females that received a CB from newly eclosed females laid an average of 20.7 (± 8.5, SEM, N = 15) eggs during the three day period. Females that were implanted with the CB from 5-day old females laid 189.5 (± 73.7, SEM, N = 15) eggs. This was significantly more than the number of eggs laid by either sham operated females or females who received the BC from newly eclosed females.

Plans: The factor present in the BC that is responsible for stimulation of oviposition will be isolated, purified and identified using a series of chromatographic and spectroscopic techniques.

IMPROVED PHEROMONE-BASED TRAPPING SYSTEMS TO MONITOR PAPAYA FRUIT FLIES (DIPTERA: TEPHRITIDAE)

R.R. Heath, N.D. Epsky, A. Jimenez[1], B.D. Dueben, P.J. Landolt, W.L. Meyer, M. Aluja[3], J. Rizzo[4], M. Camino[1], F. Jeronimo[4] and R.M. Baranowski[2]

Objectives: To develop an improved trapping system for papaya fruit flies based on a membrane formulation of the synthetic papaya fruit fly sex pheromone in cylindrical traps.

Methods: Membrane-based lures were loaded with 5, 15, 25 and 50 µl of >99% pure 2,6-MVP. The release rates from at least three lures of each load were measured every three to four days over a 23 day period to determine the change in release rate over time and the half-life of each lure. A field-test comparison of the membrane-based lures and previously used capillary lures was conducted in Homestead, Florida, using sticky-coated sphere traps. Field trials were conducted in Mexico and Guatemala to compare four trap types: 1) sticky-coated green spheres, 2) sticky-coated green opaque cylinders, 3) green opaque cylinders with internally-placed toxicant panels, and 4) dark green sticky-paper cylinders. These traps were either baited with membrane-based pheromone lures, which released ~1 µg 2,6-MVP per h, or were unbaited.

Results: A formulation method using a membrane-based system that provides a constant release rate of synthetic pheromone for the papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker was developed. Release rate measurements over 23 days indicated that lures loaded with 5, 15, 25, and 50 µl of synthetic pheromone released an average of 120, 360, 580 and 1120 ng per hr and the half-life of the lures was estimated to be 67, 184, 300 and 48 days, respectively. Field tests conducted in Mexico compared efficacy of blank and pheromone-baited sticky green spheres, cylindrical traps made from green opaque plastic that either contained a toxicant or were coated with sticky material, and cylindrical traps prepared from green sticky paper. Green opaque traps containing a toxicant and sticky paper traps captured approximately five times more papaya fruit flies than either the stick-coated green opaque traps or the sticky-coated green spheres, and presence of pheromone did not affect numbers of flies captured. Thus, the combination of the green color and the cylindrical shape provided a visual cue sufficient for papaya fruit fly capture. The pheromone lure significantly increased trap capture in similar tests conducted in Guatemala. Capture was highest in the sticky paper traps and in sticky-coated spheres. Use of the membrane-based synthetic pheromone in a cylindrical trap may provide an effective tool for monitoring papaya fruit flies.

Plans: This project has been completed.

[1]Centro de Desarrollo de Productos Bióticos, Yautepec, Morelos, Mexico
[2]Univ. of Florida, IFAS, Tropical Research and Education, Homestead, Florida
[3]Instituto de Ecología, A.C., Veracruz, Mexico
[4]APHIS-PPQ, United States Embassy Guatemala, APO Miami, Florida 34024

SYNERGISM OF THE Z11-16:AL SPECIALIST RECEPTOR NEURON OF *HELICOVERPA ZEA* (BODDIE) BY SEVERAL SEX PHEROMONES AND BRANCHED-CHAIN SYNTHETIC SEX PHEROMONE ANALOGUES

R.E. Doolittle and M.S. Mayer

Objective: To determine whether the Z11-16:Al specialist of the corn ear worm can be synergized by some of the same compounds that synergize the HS(a) Z7-12:Ac specialist receptor neuron of the cabbage looper, *Trichoplusia ni* (Hübner).

Methods: Responses of the Z11-16Al specialist receptor neuron of the corn earworm were recorded extracellularly with a sharpened tungsten electrode. The action potentials were digitized, recorded and counted by means of a computer. The stimuli chosen were admixtures of Z11-16:Al with various sex pheromones and branched chain analogues. The assay paradigm was constructed to demonstrate whether or not the mixture was synergistic and, at the same time, explore the effect of concentration.

Results: The response of the specialist neurons to Z11-16:Al was synergized by several compounds including 6-vinyldecanal and 10-vinyltetradecanal. Typically, the synergism was dependent on the concentration of both the synergist and the synergen. Other assays showed that the alcohol and acetate derivatives of these two compounds also synergized the response. Interestingly, Z7-12:Ac was a better synergist than any of the compounds assayed. Several saturated and unsaturated hydrocarbons were assayed to establish the pharmacological bounds of the synergism. None of these compounds synergized the response.

Plans: Other synthetic analogues, sex pheromone components, and host plant compounds will be evaluated to discover synergists that are active at lower stimulus intensities and to determine the range of active synergistic compounds and the relationship of volatile host plant compounds to the synergism.

VISUAL CUE AND CHEMICAL CUE INTERACTIONS IN A CYLINDRICAL DRY TRAP WITH FOOD-BASED SYNTHETIC ATTRACTANT FOR MEDITERRANEAN FRUIT FLIES

N.D. Epsky, R.R. Heath, A. Guzman[1] and W.L. Meyer[2]

Objective: A dry plastic insect trap that uses a painted band as a visual cue and baited with a two component food-based synthetic lure as a chemical cue was developed for use in monitoring populations of tephritid fruit flies. Studies were conducted to determine the effect of modifications of the painted trap body on medfly capture, and to test a new membrane-based formulation of putrescine, one of the components of the synthetic lure.

Methods: Field trials were conducted in a mix of orange trees and coffee bushes located near Palin, Guatemala. In experiment one, treatments were traps that were green or orange and had shiny or dull exteriors. Experiment two was run concurrently with experiment one and used green or orange traps with a dull exterior and three holes or six access holes. Treatments in experiment three were medium dose ammonium acetate plus 1) standard putrescine lure, 2) 3 mm putrescine lure, 3) 5 mm putrescine lure and 4) no putrescine lure. These treatments were tested in both green traps with shiny exteriors and green traps with dull exteriors. In experiment four, treatments were traps with a dull exterior baited with medium dose of synthetic lure and with visual cue widths of 3, 7.5, 12 or 15 cm. These tests were conducted in green traps and orange traps.

Results: Variation in the width of the visual cue and in the number of access holes had less effect on capture of males than on females, and capture of males was the lowest in orange traps with shiny exteriors. For both trap exterior types, the fewest flies were captured in traps without putrescine. The 5 mm putrescine lure in the shiny traps and the 3 mm putrescine lure in the dull traps captured more medflies than the standard (polypropylene vial) formulation. The width of the visual cue affected percentage of flies captured in both the green and the orange traps. When presented with a green visual cue, females preferred the widths of 12-15 cm, while males were captured equally among traps with 7.5-15 cm. Among the traps with an orange visual cue, the 7.5 cm visual cue width was better than or equal to the other widths for capture of all of the flies. The highest capture of female medflies was in green three-hole traps with dull exteriors and with 12-15 cm wide visual cues. There were interactions between visual cues associated with the trap body and the dose of putrescine, thus the optimal dose of putrescine may vary with the visual cue used in the trap design.

Plans: The results of this study suggest that solid color material could be used in place of the painted material for the trap body because attraction of females was increased when the width of the green visual cue on the trap body was increased. Testing of traps made from a solid color material have been initiated,

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[1]APHIS-IS, United States Embassy Guatemala, APO Miami, Florida
[2]Univ. of Florida, IFAS, Tropical Research and Education, Homestead, Florida
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CAPTURE OF MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) USING COLOR INSERTS IN TRIMEDLURE-BAITED JACKSON TRAPS

N.D. Epsky, R.R. Heath, G. Uchida,[1] A. Guzman,[2] J. Rizzo,[3] R. Vargas[4] and F. Jeronimo[3]

Objective: To determine if use of a color insert would improve the number of Mediterranean fruit flies, *Ceratitis capitata,* captured in trimedlure-baited Jackson traps.

Methods: A commercially produced adhesive paper was compared with the standard white inserts with manually applied sticky material in trimedlure-baited Jackson traps. Colors tested included fluorescent orange, fluorescent yellow, fluorescent light green, dark green, black and white. Field trials of native populations of Mediterranean fruit flies were conducted in Guatemala (experiments 1, 2 and 3) and in Hawaii (experiment 4). Experiments 1 & 3 in orange that was in fruit (experiment 1) or beginning to fruit (experiment 3). Experiment 2 & 4 were conducted in coffee. Most of the coffee berries had been harvested and were not present in the field during experiment 2. The coffee was in fruit during experiment 4.

Results: In experiment one, individual traps captured 0-140 males per week, and no females were captured. All traps with inserts made from the adhesive paper captured a greater percentage of flies than traps with standard inserts. Fluorescent colors captured slightly more flies than non-fluorescent colors, but the differences were not significant. In experiment 2, with a low fruit fly population, individual traps captured 0-13 flies per week. Capture was greatest when the light green insert was used. There were no differences in capture among the traps with other inserts, but in 3 of the weeks, no flies were captured in traps with standard inserts. A few females were captured during the study. Females were recovered from all trap insert types, and there were no significant differences in capture among insert types. Numbers of males trapped remained low and insert type did not affect capture in experiment 3. In experiment 4, male capture ranged from 11-466 male flies per trap. The highest capture was in traps with the fluorescent colored inserts. There were no differences among the non-fluorescent inserts and the standard insert. Differences in host species, host fruit availability and fruit fly population level among the various trials may have influenced the role of color cues in the capture of male flies by trimedlure-baited traps.

Plans: Research will be continued on the color choice by male Mediterranean fruit flies on traps baited with food-based attractants.

[1]Hawaii Department of Agriculture, Kahului, Hawaii
[2]APHIS-IS, United States Embassy Guatemala, APO Miami, Florida
[3]APHIS-Methods, United States Embassy Guatemala, APO Miami, Florida
[4]Tropical Fruit and Vegetable Research Lab., USDA-ARS, Honolulu, Hawaii

FIELD EVALUATION OF THE FEMALE MEDFLY-TARGETED DRY TRAP WITH FOOD-BASED SYNTHETIC ATTRACTANT IN NINE COUNTRIES

R.R. Heath, N.D. Epsky and J. Hendrichs[1,2]

Objectives: Studies were conducted in nine countries under an FAO/IAEA Coordinated Research Program to obtain data on the efficacy of the dry trap baited with synthetic food-based attractant for practical use in medfly Sterile Insect Technique eradication or control programs. Capture in the female-targeted trap was compared with capture in trimedlure-baited Jackson trap, which is the standard male-targeted medfly trap.

Methods: A standard protocol was developed and followed by cooperators in Spain, Greece, Morocco, Turkey, Guatemala, Argentina, Costa Rica, Mexico and Honduras. Tests were conducted in at least two sites in each country, with ten dry traps with food-based synthetic attractant and ten trimedlure-baited Jackson traps tested at each site. Traps were placed in a line or block with alternating trap types. Traps were rotated sequentially after each sample. All lines or blocks within a site were placed in similar hosts where possible. Each of these tests was to be run for 8 weeks and trap data collected twice a week.

Results: Trap capture varied depending on the population densities and the country of the test (Table 1). The female-targeted traps captured from 5 - 80% of the total capture in the Jackson traps. However, 50 - 90% of the medflies captured were female. Less than 3% of the medflies captured in the Jackson traps were females.

Plans: Tests will be expanded compare the dry trap tested herein with a modified dry trap that is more user-friendly and efficacious. These traps will be compared with trimedlure-baited Jackson traps, a McPhail trap baited with liquid protein solution, and a McPhail trap or other local trap baited with the food-based synthetic attractant.

Table 1. Number of flies per trap per day

Country	Jackson Trap		Dry Trap with Food-based Lure	
	Males	Females	Males	Females
Spain	0.76	0.0	0.02	0.22
Greece	2.22	0.0	0.04	0.08
Morocco	3.26	0.02	0.18	0.29
Turkey	4.86	0.03	1.54	2.51
Guatemala	0.59	0.0	0.11	0.17
Costa Rica	0.06	0.001	0.003	0.009
Argentina	0.66	0.007	0.08	0.29
Mexico	2.44	0.07	0.02	0.06
Honduras	0.35	0.23	0.03	0.04

[1]Insect and Pest Control Section, International Atomic Energy Agency, Vienna, Austria
[2]Partial support for this research is provided by FAO/IAEA

HOST PLANT EFFECTS ON PHEROMONE PRODUCTION BY FEMALE CABBAGE LOOPERS

R.R. Heath, B.D. Dueben and R.L. Abernathy

Objectives: To determine if the production of pheromone in female cabbage loopers is affected by the presence of a host plant under greenhouse conditions. The amount of pheromone released as volatile emissions was evaluated and the response of males to the emissions was monitored to determine if males showed a preference for females on host plants.

Methods: Cabbage looper pupae were obtained from a maintained laboratory colony. Sexes were held separately in naturally lighted, vented areas in a greenhouse and were tested 3-4 days after eclosion. Cotton plants (Germaine 510) were grown from seed in a greenhouse and were tested at intact plants at 5-8 weeks post germination. Pheromone was collected from 5 females in the presence of cotton and 5 held alone beginning at 18:00 and continuing at 2 hr intervals until 10:00 the following morning using an automated volatile collection system. Simultaneously, a portion of the volatiles being collected was routed to a wind tunnel to test real time biological response of males to the female pheromone being produced. Tests were conducted over a one year period. Volatile collections were analyzed for the major pheromone component Z7-12:Acetate using capillary gas chromatography.

Results: In early summer, there appeared to be a difference in both the amount and time of pheromone release. Females in the presence of cotton produced more total pheromone than those held alone and their peak pheromone production was at the time that correlated with peak make response (Fig. 1). However, at other times of the year, no consistent effect of the presence of the host plant was seen.

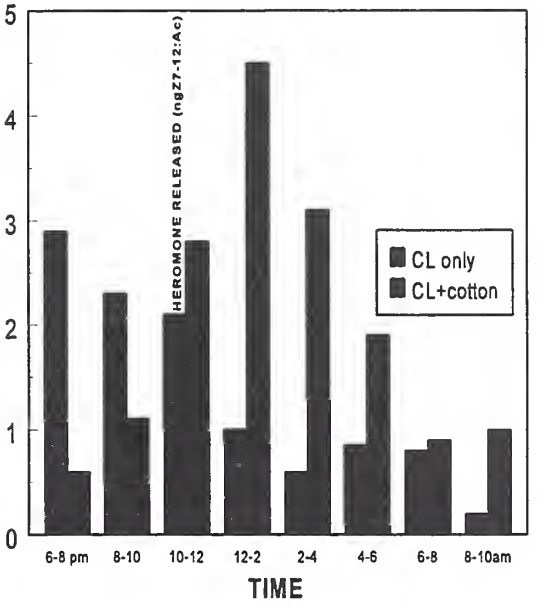

Figure 1: The amount of pheromone released from female Cabbage Loopers during a single collection in June 1995.

Plans: This study will be repeated over several summers to determine if seasonal host plant effects exist. Wild insects also will be evaluated to determine if there are differences in response with those of the laboratory reared colony.

OVIPOSITION BEHAVIOR OF *Anastrepha suspensa* (DIPTERA: TEPHRITIDAE) AND USE OF KAIROMONES IN PEST MANAGEMENT

W.L. Meyer[1], R.R. Heath, R. M. Baranowski[1], N.D. Epsky and B.D. Dueben[2]

Objectives: Laboratory and field studies were initiated to define the stage of maturity of guava selected by mated Caribbean fruit flies. Identification of visual cues and host kairomones from this stage will be pursued with the goal of improving traps and baits used for detection and monitoring of this pest.

Methods: Guava fruits at different stages of maturity were obtained from a commercial grove in Homestead, FL, shipped overnight to Gainesville, and placed in refrigeration until use. For each maturity stage, the fruit was classified by weight, size, hardness (Newtons of force to penetrate skin) and color (L*,a*,b* values). Similar fruits were placed in a wind-tunnel bioassay and oviposition-deprived flies from either fruit-reared and artificial diet-reared laboratory colonies were allowed to respond to the visual and odor cues emitted by the fruit. Fruits that were scored positively for oviposition (ovipositor inserted into fruit and host-marking behavior observed after removal of ovipositor) and oviposition attempts (with no host-marking behavlor observed) were removed from the flight tunnel, placed in containers containing vermiculite and held in a growth chamber to determine success of larval development. The stage of maturity of the fruit used by females was recorded. In a mixed -variety guava grove located at the Tropical Research and Education Center in Homestead, FL, small (<4cm diameter) guava fruits were bagged with paper bags and were exposed at different stages of maturity to natural oviposition by Caribbean fruit fly for 3-5 days. The fruits were then re-bagged and allowed to ripen on the tree. After ripening, they were brought into the laboratory and placed in containers with vermiculite. Success of larval development was assessed by the number of pupae produced and the number of adults that emerged.

Results: Preliminary results indicate that there is selection by the female (reared on artificial diet) when presented a choice of fruits at different stages of maturity for oviposition. In the field studies, bagging the fruit caused abnormal (i.e. premature) ripening in many instances.

Plans: Further study is needed to determine the ovipositional activity period of the fruit-reared flies in the laboratory. In the field studies, alternative bagging protocols will be evaluated to overcome problems encountered with premature fruit ripening.

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[1]Dept. of Entomology and Nematology, University of Florida.

[2]Partial support for this research is provided by the Florida Dept. of Agric. & Consumer Services, Tropical Fruits Advisory Council.

SYNTHESIS AND TURN-OVER RATES OF VOLATILES RELEASED FROM HERBIVORE-INJURED COTTON

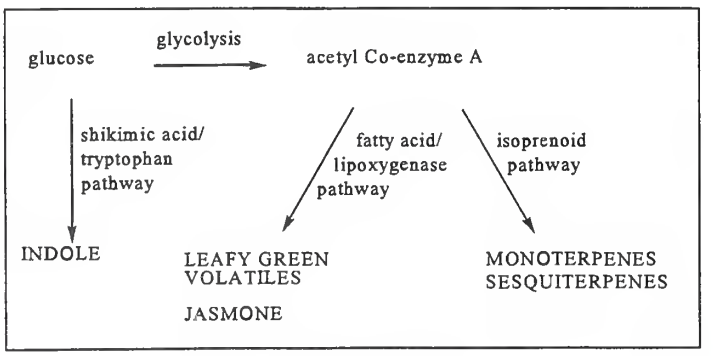
P.W. Paré and J.H. Tumlinson

Objectives: Determine how insect feeding affects biosynthesis of terpenes and other volatiles released by insect herbivore injured cotton to identify points of regulation in the activation of this plant alarm mechanism.

Methods: Cotton *(Gossypium hirsutum* L. cv. Delta Pineland 90), plants were grown in a greenhouse designed to exclude unwanted insects in a potting soil/vermiculite mixture for six weeks until they were approximately 25-30 cm tall and had not set flower buds. Natural light was supplemented with 400 Watt sodium lamps on a 16 hour light / 8 hour dark photoperiod; temperature was maintained at 32 ± 5 C with a relative humidity of 85 ± 10%. Five second instar beet armyworm (*Spodoptera exigua* Hübner) larvae, collected at 08:00 hours and starved until 15:00 hours were placed on each plant inside a glass volatile collection chamber. Charcoal purified air was passed down over the plant and a fraction of the air stream was drawn off by vacuum through a filter trap containing polymeric adsorbent (Super Q). Volatiles collected from the plant were eluted from the filters with dichloromethane and analyze by capillary gas chromatography and by GC-mass spectroscopy.

Results: Twenty-two volatile compounds have been collected and identified from insect-damaged cotton can be divided into three biosynthetically distinct classes: monoterpenes and sesquiterpenes of the isoprenoid pathway, green leafy volatiles and butyrates of the fatty acid/lipoxygenase pathway and indole of the shikimic acid/tryptophan pathway. Our results indicate that insect feeding activates the synthesis and release of at least one metabolite of the shikimic acid pathway, indole, and a select group of monoterpenes and sesquiterpenes of the isoprenoid pathway. The terpenes appear to be a mix of constitutive compounds which are released at the time of insect wounding to the leaves and induced compounds which are released only after a delay of up to sixteen hours. Examples of constitutive compounds include the monoterpenes α-pinene and β-pinene as well as the sesquiterpene caryophyllene. At least some fraction of the monoterpenes (*E*)-β-ocimene, myrcene, and limonene appears to by synthesized *de novo*. This is also the case with the sesquiterpenes (*E,E*)-α-farnesene and (*E*)-β-farnesene, α-humulene and the homoterpenes (3*E*)-dimethyl-1,3,7-nonatriene and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

Plans: Experiments are being conducted to determine how artificial damage and beet armyworm spit applied exogenously to wounded leaves affect the synthesis and release of cotton volatiles.



ATTRACTION OF THE SPECIALIST PARASITOID *MICROPLITIS CROCEIPES* TO SYSTEMICALLY RELEASED VOLATILES FROM UNDAMAGED COTTON LEAVES OF CATERPILLAR DAMAGED PLANTS

U.S.R. Röse and J.H. Tumlinson

Objective: 1. To investigate whether parasitoids are attracted to systemically released cotton volatiles released from undamaged top leaves of a cotton plant that is damaged by caterpillars on the lower leaves only 2. To determine how wasps respond to manually damaged plants.

Method: For the induction of a systemic response, starved third-instar larvae of beet armyworm, *Spodoptera exigua*, were caged and replaced each day on the lower leaves of a cotton plant (8 leaves), *Gossypium hirsutum*, var. "Delta Pine 90". The undamaged top leaves of the damaged plant were enclosed in a glass sleeve of the volatile collection chamber developed by R.R. Heath and A. Manukian. After four days volatiles were collected from the top leaves of the systemic cotton and top leaves of a control for one hour prior to flight tunnel experiments, from 1100-1200 hours and after the flight tunnel experiment from 1400-1500 hours. The experiments were conducted in the time of maximum release of induced compounds in cotton, determined in earlier experiments. All parasitoids were given a preflight experience by allowing them to sting an artificial diet fed host larvae immediately prior to the release in the flight tunnel. Experiments were carried out as two choice experiments, comparing an undamaged control plant with a plant that released induced volatiles systemically or comparing an undamaged control plant with an manually damaged plant For manual damage, top leaves of cotton plants were punched with a garlic press at 1200 hours when flight tunnel experiments were started and again after one hour, to ensure continues volatile release. Volatiles were only collected after the flight tunnel experiment from 1400-1500 hours. Volatiles were collected on volatile collection filter, containing 25mg Super Q as an adsorbent. Volatiles were extracted from the filters by washing them with Methylene Chloride. Extracts were analyzed by capillary gas chromatography and GC-mass spectroscopy.

Results: Wasps given a choice between plants systemically releasing volatiles and control plants responded to the odor sources with 77 wasps of a total of 100 wasps. Wasps chose plants that released volatiles systemically, significantly more often than undamaged control plants. Only 7 of 40 wasps responded to odors from a manually damaged plant and a control plant. The choice for either plant was less clear. However, wasps are significantly less attracted to odors from manually damaged plants compared to plants that released caterpillar induced volatiles systemically.

Plans: The study of attraction of parasitoids to the systemic compounds in cotton and manually damaged plants will be completed, and compared to a generalist parasitoid.

HOST SPECIFIC PLANT COMPOUNDS IN FRASS VOLATILES - SHORT RANGE HOST RECOGNITION BY THE SPECIALIST ENDOPARASITOID *MICROPLITIS CROCEIPES*

U.S.R. Röse and J.H. Tumlinson

Objective: 1. To investigate whether parasitic wasps can distinguish between frass from different caterpillars in the flight tunnel, when given a choice between host and non host frass. 2. How experience effects the flight behavior, 3. How diet of host larvae effects the ability of the parasitoid to distinguish between hosts and non-hosts.

Method: In all experiments caterpillar frass was collected from 3rd instar lepidoptera larvae, previously fed on cotton plants, *Gossypium hirsutum*, nectaried var. "Delta Pine 90" for two days or on pinto bean diet. The frass used for wind tunnel experiments was less than 3 hours old. Wind tunnel experiments were conducted as two choice experiments, each experiment comparing fresh host (*Helicoverpa zea*) and non host frass (*Spodoptera frugiperda, S. exigua*) as a volatile source. Wasps were released 80 cm downwind of the volatile sources, and given three chances to complete a flight to either source. Preflight experienced was given A) on host frass obtained from cotton fed *H. zea* larvae, B) host frass obtained from artificial diet fed *H. zea* larvae, C) by allowing the wasps to sting a diet fed host larvae, or D) wasps were not given any preflight experience (naive wasps).

Results: The specialist parasitoid *M. croceipes* was able to distinguish in flight by means of frass volatiles, whether the frass was from a host or non-host lepidoptera species. However, the parasitoids were only able to distinguish between host (*H. zea*) and non-host (*S. exigua*, *S. frugiperda*) frass volatiles when the larvae were fed on cotton, but not when larvae were fed on pinto bean diet. Therefore, a host modified plant compound in host frass appeared to guide the parasitoid to its host. Wasps that were naive, or were allowed to sting a diet fed host larvae were not able to distinguish between frass volatiles of cotton fed host and non-host larvae. Parasitoids that were experienced on pinto bean diet host frass were able to distinguish between *H. zea* and *S. exigua*, but not between *H. zea* and *S. frugiperda*. Experience appeared to play a major role in the successful location of host frass volatiles.

Plans: There are no further plans on this project.

INDUCTION OF PHEROMONE PRODUCTION IN FEMALES OF *HELIOTHIS VIRESCENS* BY TOPICAL APPLICATION OF AN AMPHIPHYLLIC PSEUDO TETRAPEPTIDE ANALOG OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDE

P.E.A. Teal, R.J. Nachman[1], R.L. Abernathy and J.A. Meredith

Objectives: To design and develop synthetic analogues of insect neuropeptides that penetrate the insect cuticle and maintain bioactivity.

Methods: A pseudotetrapeptide analog of the C-terminal active core (FSPRLamide) of pheromone biosynthesis activating neuropeptide (PBAN) was synthesized by replacing the amine terminal two amino acids with an *o*-carboranyl-threonine moiety. Pheromonotropic activity of the analog was assessed in injection bioassays in which females of *Heliothis virescens* were injected with different doses of the analog or PBAN. Females were incubated for 1 h after injection and then the sex pheromone glands were excised and extracted in hexane containing internal standards. The extracts were then analyzed by capillary gas chromatography to determine the amount of pheromone present. Topical application studies were conducted by applying various doses of the pseudotetrapeptide analog or PBAN to the descalled abdomen in water. For these studies females were incubated for 1 h prior to extraction of the pheromone glands. Temporal activity studies for topically applied compounds were conducted by application of 100 pmol of the analog or PBAN to the descalled abdomen of females. Females were incubated for periods of time from between 15 min to 7 h after application. After incubation the sex pheromone glands were excised, extracted and the extracts analyzed for the amount of pheromone present as above.

Results: The pseudotetrapeptide analog was 10 fold more potent than PBAN in stimulating pheromone production when injected into females of *H. virescens*. Thus, 0.5 pmol of the pseudotetrapeptide analog stimulated production of as much pheromone as did 5.0 pmol of synthetic PBAN. Additionally, the pseudotetrapeptide stimulated pheromone production when applied topically in water to the abdomen. The amount of pheromone present in extracts increased in a linear fashion when doses of between 0.5 - 50 pmol were applied and remained constant at doses from 50 - 200 pmol. PBAN did not stimulate production of pheromone when applied topically at any dose. Temporal response studies indicated that pheromone production was maintained for periods of up to 7 h after topical application of the pseudotetrapeptide analog.

Plans: Other pseudopeptide analogues will be designed, synthesized and tested for pheromonotropic activity in topical application bioassays. Modifications will be designed so as to impart an amphiphyllic character to the peptide, to facilitate penetration of the cuticle, and so that the compounds resist enzymatic degradation after entering the hemolymph.

[1]USDA/ARS, College Station, TX.

ISOLATION AND IDENTIFICATION OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDES FROM *HELIOTHIS VIRESCENS*

P.E.A. Teal, J.A. Meredith and J.H. Tumlinson

Objectives: To isolate and identify pheromonotropic neuropeptides from the nervous system of *Heliothis virescens*

Methods: The heads of females of *H. virescens* were excised and extracted with acetone prior to homogenization in aqueous 0.1% trifluroacetic acid. The extracts were bioassayed for pheromonotropic activity by injection of extracts into females of *H. virescens* during the photophase, when pheromone is not normally produced. After injection females were incubated for one hour prior to extraction of the pheromone gland and analysis of the extract to determine the amount of pheromone present by capillary gas chromatography. The extracts were subjected to solid phase extraction using cation exchange and reversed phase systems. Active fractions from solid phase extraction were subjected to HPLC separation using a four different protocols. Material from each separation was subjected to pheromonotropic bioassays prior to subsequent chromatographic separations. Purified fractions were subjected to capillary zone electrophoresis, mass spectral, amino acid and sequence analyses.

Results: To date, three peptides have been purified to homogeneity and subjected to structural analyses. Mass spectral analysis of the most potent peptide indicated an accurate mass of 3932.02 and amino acid analysis indicated the presence of 40 amino acids. Sequence analysis of this peptide failed, indicating that it is amine terminally blocked. The second peptide had a mass of 3740, contained 37 amino acids and sequence analysis resulted in identification of the amine terminal 20 residues. Mass spectral analysis of the third peptide indicated a molecular weight of 1951.35.

Plans: The complete structures of the pheromonotropic peptides isolated from the cephalic ganglia will be elucidated using a combination of amino acid analysis, protein sequencing and mass spectral techniques coupled with enzymatic cleavage. Proof of structure and activity will be obtained by synthesis of the peptides coupled with bioassays of the synthetic material.

ISOLATION AND IDENTIFICATION OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDES FROM *MAMESTRA BRASSICA*

P.E.A. Teal, A. Fonagy and J.H. Tumlinson.

Objectives: To isolate and identify pheromonotropic neuropeptides from the cephalic ganglia of *Mamestra brassica*.

Methods: The subesophageal ganglia were dissected from the heads of adult females and homogenized and extracted in aqueous 0.1M acetic acid. The extracts were bioassayed for pheromonotropic activity by injection into females. After injection, females were incubated for one hour prior to extraction of the pheromone gland and analysis of the extract to determine the amount of pheromone present by capillary gas chromatography. The extracts were subjected to solid phase extraction using ion exchange and reversed phase systems. Active fractions from solid phase extraction were subjected to semipreparative HPLC separation using a reversed phase column. Active areas from the semipreparative HPLC separations were subjected to analytical HPLC separations using reversed phase and inversed gradient reversed phase protocols with fractions from each separation being assessed for bioactivity. Final purification was accomplished via microbore HPLC using a reversed phase column.

Results: To date, one peptide has been purified to homogeneity and subjected to structural analyses. Mass spectral analysis of this peptide indicated a mass of 2136.06. One other peptide has been purified by analytical HPLC but no structural information has been obtained.

Plans: The complete structure of the pheromonotropic peptides will be elucidated using a combination of amino acid analysis, protein sequencing and mass spectral techniques. Proof of structure and activity will be obtained by synthesis of the peptides coupled with bioassays of the synthetic material.

IMPORTED FIRE ANT

AND

HOUSEHOLD INSECTS

CRIS - 6615-32000-026-00D -- Integrated Control of Insect Pests in an Urban Environment with Emphasis on Roaches, Fleas and Ants

CRIS - 6615-32000-028-00D -- Fire Ant Ecology and Management

CRIS - 6615-32000-029-00D -- Biological Control of Fire Ants

DEVELOPING REDUCED-RISK STRATEGIES FOR INTEGRATED MANAGEMENT OF PESTS AND ARTHROPOD-BORNE DISEASES USING SPATIALLY-BASED RISK ASSESSMENT AND PRECISION TARGETING METHODS [1]

R J. Brenner, D.A. Focks, D.F. Williams, S.M.Valles, R.T. Arbogast, D.K. Weaver, and P.G. Koehler

Objectives: It is important to develop of Integrated Pest Management (IPM) that reduces the amounts of pesticides used, while reducing risks associated with pests and disease vectors. Scientists at the CMAVE, cooperators elsewhere in ARS, Univ. of Florida, US ARMY CHPPM, EPA, and private companies, have established a 4-year project to address these needs for the Department of Defense, and the private sector. DoD recently has published its definition and multi-step process of IPM for DoD facilities, that will require the following: (1) routine monitoring for preventing pests and disease vectors from causing unacceptable damage, (2) determining the risks associated with pests and disease vectors, (3) selecting the least-toxic strategy when intervention is warranted, (4)targeting interventions, (5) documenting actions, and (6) verifying that IPM processes are used. Arthropod-related disease and pest problems are not uniformly distributed in space or time. Obviously, the ability to target interventions to correspond to the spatial and temporal distributions of pests would not only reduce the direct costs of control but would reduce potential environmental and health consequences of the mitigation efforts as well.

Methods: Currently, there is no quantitative procedure for determining spatial distribution of disease vectors and pests, and attendant risks, or for selecting interventions that maximize risk reductions including risks associated with pollution from pesticides. Our methods will reduce pesticide use and risks using "precision targeting" in comparative risk assessment & comparative risk reduction processes. These will be done in a manner that *standardizes* the process so that it is comprehensive (applicable for virtually any invertebrate, vertebrate, and plant pests,) *verifiable* (will provide measures of efficacy), *documentable*(will provide useful records through both space and time), and *portable* so that existing DoD Global Position Systems (GPS) and Geographic Information Systems (GIS) can integrate our proposed programs and be immediately functional in DoD settings

Results: New Project.

Plans: Field studies are underway in which precision targeting is used to identify and subsequently, treat Pharaoh ant foci at the Riverside BOQ at NAS-JAX. This study will document the increase in efficacy, the reduction in pesticides, and reduction in risks from pesticides that result from this strategy. Studies also are underway to provide simple, inexpensive, standardized monitoring techniques for other selected pests, including cockroaches, fire ants and stored-products pests. Field studies will be conducted in cooperation with the private sector, and with DoD personnel at DoD facilities.

Additional cooperative research is under way with US ARMY CHPPM entomologists, and private sector companies in which heat treatments will be adapted for use in precision targeting applications within warehouses, food production facilities, and DoD housing.

Also, risk assessment processes are being developed with data pertaining to dengue fever from DoD installations, and the impact of traditional treatment strategies on risk reduction, and pollution prevention.

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[1] This research is partially funded under the Strategic Environmental Research and Development Program (DoD, EPA, DOE) under the Pollution Prevention section; project no. PP-1053, and under an Interagency Agreement between EPA (Biopesticides and Pollution Prevention Division) and USDA-ARS, in support of the EPA-DoD Memorandum of Agreement on reducing pesticide use.

MEASURING SPATIAL DISPLACEMENT OF *BLATTELLA GERMANICA* (L.) (BLATTARIA:*BLATTELLIDAE*) POPULATIONS PRESSURED BY REPELLENT-TREATED HARBORAGES [1]

R.J. Brenner, D.E. Milne, K.M Kinscherf[2] & T.F. Connors[2]

Objectives: Cockroaches can cause serious allergies to humans, and have the potential to spread germs and filth to the surfaces they contact. Specific objectives of this study included (a) the development of testing procedures for evaluating changes in spatial distribution of German cockroaches (Orlando normal strain) subjected to repellents, and (b) an evaluation of the efficacy of 2% n-methyl neodecanamide (MNDA) Ajax cleaner formulations in displacing German cockroaches from established habitats under continuous or cycled lighting.

Methods: Trials involved 16 harborages (4x4 grid) consisting of yellow-laminated Plexiglas and floor tiles (58 cm^2) in 1.2 x 1.2 m arenas, and concurrent studies involving 49 harborages (7x7 grid) in an adjacent 6 x 8 m room. Harborage design allowed assessment of population spatial distributions without disturbing resting cockroaches. Studies were conducted either with a 12L:12D cycle, or under continuous lighting. Spatial statistical analysis (kriging) was used to compare and quantify the distribution of German cockroaches among harborages. Cockroaches were collected, and 0.63 g of the repellent solution was applied to harborages encompassing at least 85% of the cumulative distribution. All other harborages received an equal volume of water. Cockroaches were then reintroduced into the arenas or room, and their distribution relative to the repellent-treated and water-treated harborages was monitored for 20 or 28 days.

Results: Results in arenas and rooms under either cycled or continuous lighting revealed remarkably similar results. Subsequent spatial analysis revealed a dramatic shift of 96-98% of the reintroduced cockroach populations to non-repellent treated harborages; patterns persisted for the duration of the study (28 d (12:12) and 20 days (continuous lighting, Fig. 1)). Spatial analysis also was used to quantify the areas of emigration and immigration resulting from the repellent; 89% of the population was redistributed into 16.7 m^2, leaving 11% of the population in the remaining 28.5 m^2, with only 2-4% of the population remaining in the 16.1 m^2 that originally contained 89% of the cockroach population. This approach, therefore, allows researchers to quantify the impact of an intervention that changes distribution, rather than survivorship. These studies indicated that this formulation clearly precluded populations from re-establishing in previously inhabited harborages under either lighting condition. These spatial analysis techniques will be useful to scientists in measuring risks associated with pests, and in determining practical expectations for using repellents to prevent infestations in homes and to safeguard critical areas from cockroach contamination.

Plans: A manuscript has been submitted to Environmental Entomology. Additional research is underway to evaluate efficacy in reducing foraging areas on counter tops and appliances in simulated kitchens. Studies are planned to use repellents and baits in a push-pull reduced risk strategy at DoD facilities under the SERDP project titled "Pesticide reduction through precision targeting".

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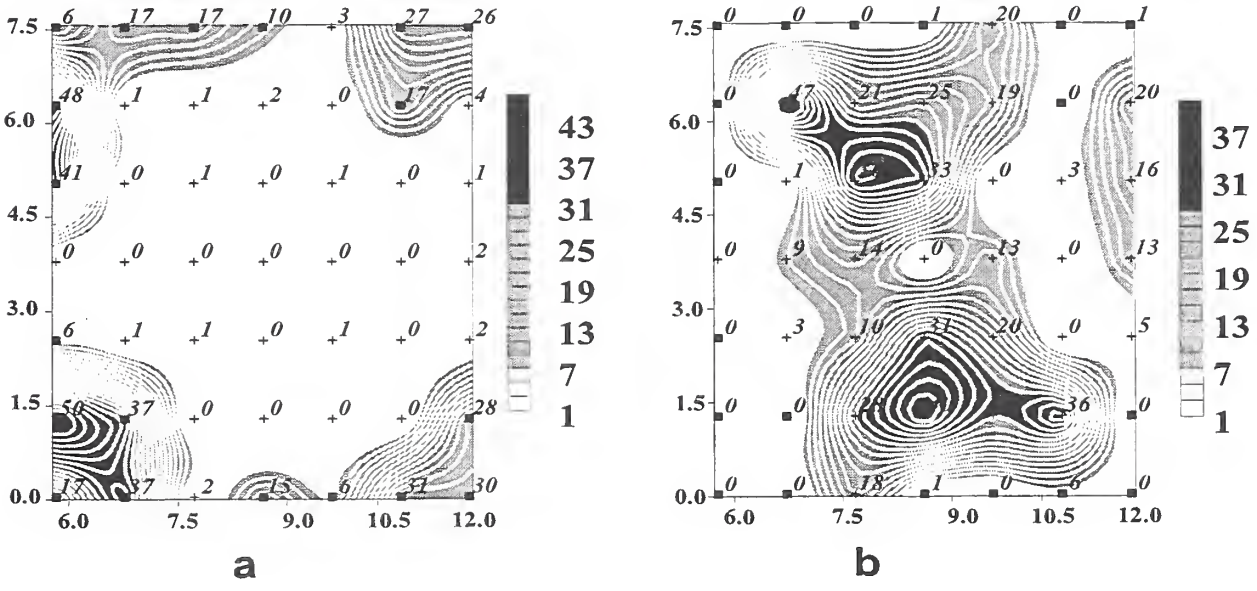
[1] This research was conducted under Cooperative Research And Development Agreement No. 58-3K95-4-227 between the Agricultural Research Service and Colgate-Palmolive Company.

2 Colgate-Palmolive Company Corporate Technology, Household Surface Care R & D, Piscataway, NJ 08854

Fig. 1. Spatial distribution of German cockroaches under continuous lighting in the east room once stasis had occurred (panel a, pretreatment), and at the post treatment time of 20 days (b). Contours represent isolines of equal cockroach density. In panel **a**, dark squares indicate those selected for repellent treatment based on these data, and harborages selected for water treatment are represented by crosses. Number above harborage is number of cockroaches on that day. X and Y axis are in meters.



SIMULATION ANALYSIS OF POTENTIAL DENGUE FEVER RISK UNDER GLOBAL CLIMATE CHANGE SCENARIOS

D.A. Focks, T.H. Jetten[1], W.J.M. Martens[2] and J.A. Patz[3]

Objectives: To attempt a global assessment of the potential impacts of anthropogenically-induced climate change on the mosquito-borne human illness, dengue hemorrhagic fever.

Methods: The purpose of the present work was to evaluate the influence of warming temperatures on the intensity and distribution of dengue transmission throughout the world using an expression of vectorial capacity (*VC*) modified to reflect the role of temperature on development and survival of the vector and virus. In malaria and dengue epidemiology, the concept of vectorial capacity is used to express how fast an epidemic may develop if an infectious case is introduced. It can be defined functionally as the mean number of potentially infective contacts made by a mosquito population per infectious person per unit time and is therefore influenced strongly by the characteristics of the mosquito population. In our work we defined it as-

$$VC = m\ b\ c\ a^2 (P^n / -Ln\ P)$$

where *a* is the number of bites per man per day, *b* is the probability that an infectious mosquito transmits dengue while biting a susceptible human being, *c* is the probability that a mosquito acquires a dengue infection while biting human beings with infective parasites, *m* is the number of female mosquitoes per person, *n* is the duration of the extrinsic incubation period, *P* is the survival rate of the mosquito and VC is the vectorial capacity. This expression was modified to reflect the influence of temperature on adult mosquito survival, the lengths of the gonotrophic cycle and the extrinsic incubation period of the virus in the vector, and vector size and then used to predict dengue transmission at >1,000 sites around the world for each week of the year using current weather *plus* 0, 2, or 4°C.

Results: The technique was validated by successfully comparing model projections and the observed spatial, temporal, and altitudinal distribution of dengue using current climate in 5 cities that are endemic or have had epidemics in the past. Results indicate that the current warming projection of the Intergovernmental Panel on Climate Change of 2°C by the end of the next century can be expected to result in an increase in the latitudinal and altitudinal range of dengue; the duration of the transmission season will also increase in temperate locations as well. Finally, the results indicate that climate-related increases in transmission intensity will result in significant increases in the incidence of dengue hemorrhagic fever and shock syndrome among infants and adolescents.

Plans: Manuscripts describing various facets of this work have been submitted to and accepted by Climate Change, Journal of the American Society of Tropical Medicine and Hygiene, and Journal of the American Medical Association.

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[1] Graduate School for Production Ecology, Wageningen Agricultural University, Wageningen, The Netherlands.
[2] Department of Mathematics, Universtiy of Limburg, Maastricht, The Netherlands.
[3] Johns Hopkins School of Public Health, Johns Hopkins University, Baltimore, MD.

IMIDACLOPRID AS A SYNERGIST FOR FUNGAL DISEASES OF GERMAN COCKROACHES (DICTYOPTERA: BLATTELLIDAE)

W.T. Grush[1], P.G. Koehler[1], and R.J. Brenner

Objectives: To evaluate the potential of imidacloprid, a chloronicotinile insecticide, as a synergist for fungal diseases of German cockroaches.

Methods: Technical imidacloprid (Bay NTN 33893, 97.4% A.I.; Miles, Kansas City, Missouri) was dissolved in acetone at 0.1 µg/µl. *Beauveria bassiana* (Mycotrol WP; 63% A.I.; Mycotech Corporation Butte, Montana) was diluted at a rate of 6.2 mg in 3 mL of water. Male Village Green cockroaches were collected from rearing containers and placed in glass Mason jars (500 mL) with one of four treatments: imidacloprid, *Beauveria*, imidacloprid and *Beauveria*, or untreated control. Imidacloprid was applied topically to 10 cockroaches (0.1 µg/insect). *Beauveria* treatments were applied by pipetting 3 mL of suspension into a glass jar and rotating until dry. Both imidacloprid and *Beauveria* treatments were done by placing cockroaches topically treated with imidacloprid into jars treated with *Beauveria*. Controls were 10 untreated cockroaches placed into jars treated with water. Each treatment was replicated five times; cockroaches were provided with food and water during the experiment. Mortality was recorded at 11 days. The data were analyzed by analysis of variance and means separated by Tukey's Studentized Range (HSD) Test [P = 0.05; SAS Institute 1988].

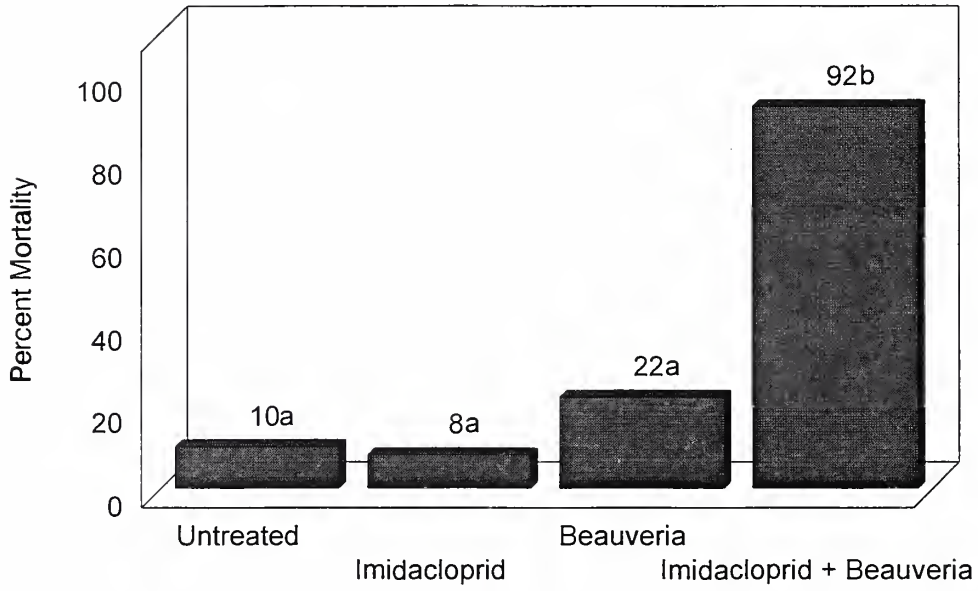
Results: Topical applications of 0.1 µg imidacloprid per insect or *Beauveria* residues of 11.23 ug/cm^2 did not result in significant mortality of German cockroaches. At 11 days, mortality of untreated controls was 10%, imidacloprid was 8%, and *Beauveria* was 22%. However, treatment with both imidacloprid and *Beauveria* resulted in 92% mortality at 11 days. Imidacloprid is not highly toxic to German cockroaches because it is metabolized by mixed function oxidases. However, at sublethal doses it prevents normal feeding and grooming behavior. German cockroaches primarily protect themselves from pathogens and nematodes by grooming. Therefore, after exposure to *Beauveria* spores, cockroaches treated with sublethal amounts of imidacloprid are not able to remove the spores from the surface of their cuticle. The spores can then germinate, and hyphae can penetrate the cuticle to infect and kill the cockroach. Imidacloprid can be developed for use as a synergist for fungal pathogens (e.g. *Beauveria* and *Metarhyzium*) that are not very effective for German cockroach control when used alone.

Plans: This research will be part of an M.S. Thesis.

[1] Department of Entomology and Nematology, University of Florida.

SYNERGISM OF BEAUVERIA WITH IMIDACLOPRID

GERMAN COCKROACH MORTALITY (11 day)



EVALUATION OF CAT FLEA (*CTENOCEPHALIDES FELIS*) LARVAE PROTEIN DIETS FOR OPTIMAL DEVELOPMENT AND SURVIVAL

D.L. Richman[1], P.G. Koehler[1], and R.J. Brenner

Objectives: To determine cat flea larval survival and adult development times for eight commercially available protein sources.

Materials and Methods: *Ctenocephalides felis* eggs were sifted from debris at the bottom of laboratory cat cages. Diets tested: slaughterhouse blood (fresh bovine blood collected from the University of Florida Meat Science Department, oven dried for eight hours at 49°C, air dried at 30°C, hand pulverized); spray dried enzyme hydrolyzed poultry protein; spray dried enzyme hydrolyzed liver protein; spray dried bovine blood; spray dried poultry blood; adult flea feces; brewer's yeast. Spray dried diets were commercially available (California Spray Dry, Stockton, CA). Diets were tested alone or supplemented with brewer's yeast (1:1 [wt/wt]). One flea egg and 5 mg of test diet were placed into each well of a 92-well titer plate (0.1 ml wells) covered with parafilm. For each test, there were 10 wells per diet, and each test was replicated 3 times. Adult flea feces and brewer's yeast alone were also tested.

Development times and mortality were recorded daily. Adults were frozen, oven dried, sexed, and weighed. Time to pupation and adult emergence, percent pupation, and percent adult emergence were analyzed by analysis of variance, and means separated using Scheffe's Test (P = 0.05).

Results: Adult flea feces diet, the natural diet of flea larvae, yielded the highest percent pupation (100%) and adult emergence (87%); however, adult emergences using spray dried bovine (79%) and poultry (66%) blood were not significantly lower. Percent pupation and adult emergence using liver protein were significantly lower than the other successful diets. All other diets (slaughterhouse blood, spray dried poultry protein, and brewer's yeast) were unsuccessful in producing adult fleas.

Yeast supplementation improved pupation and adult emergence for fleas reared on the slaughterhouse blood diet from 0% to 55% pupation and from 0% to 42% adult emergence.

Time to adult emergence did not differ significantly for any of the tested diets. Times ranged from 19.44-22.43 days.

Slaughterhouse blood supplemented with yeast produced the heaviest fleas, and adult flea feces diet produced the lightest fleas.

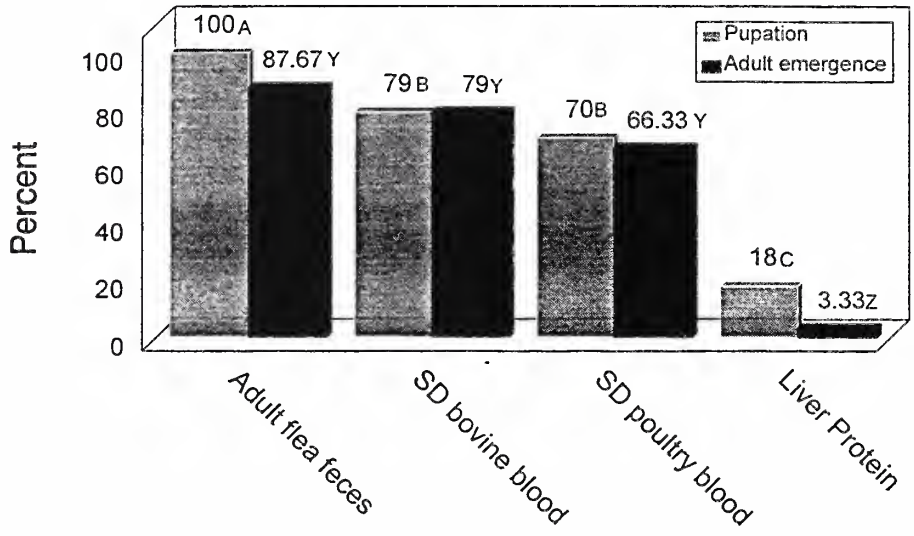
Spray dried bovine or poultry blood is a satisfactory diet for artificially reared laboratory fleas. Because it is commercially available, it eliminates the need to collect and process slaughterhouse blood, which needs to be nutritionally supplemented.

Plans: This research will be part of an M.S. thesis.

[1] Department of Entomology and Nematology, University of Florida

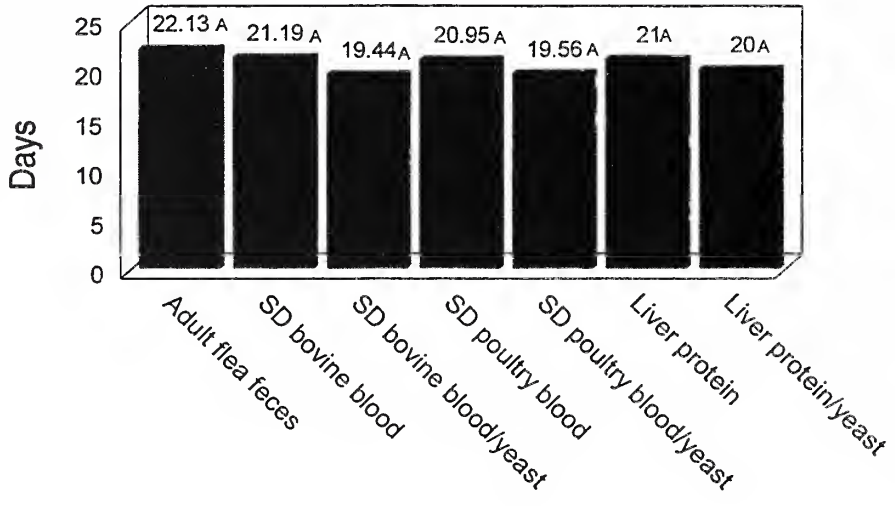
Survival of Cat Flea Larvae

Effect of Successful Non-Yeast Diets on Percent Pupation and Adult Emergence



Development Times of Cat Flea Larvae

Effect of Successful Diets on Time to Adult Emergence



DETOXIFYING ENZYME INDUCTION BY ALLELOCHEMICALS IN THE GERMAN COCKROACH

C.A. Strong, P.G. Koehler[1] and S.M. Valles

Objective: Determine if ingested allelochemicals are able to induce detoxifying enzymes in the German cockroach, *Blattella germanica* (L).

Rationale and Methods: The diet of agricultural pests often include allelochemicals which may increase the insect's tolerance to insecticides. Menthol and peppermint oil were able to induce epoxidases in the fall army worm (Yu 1982). Additionally, N-demethylases in the fall army worm were induced by limonene, carotene and sitosterol (Brattsten et al. 1977). German cockroaches are not typically exposed to plants containing allelochemicals; however, the urban environment is littered with alien compounds such as spices, cleaners and disinfectants.

Less than 1 week old, lab reared, adult male cockroaches were held in glass jars (4 l) with harborage and water. After 24 h the cockroaches were allowed to feed on ground laboratory rodent chow augmented with a candidate inducer at 0.2% for 4 d. The diet was augmented with 4 potential inducers, 3 allelochemicals and a barbital. Allelochemicals were menthol, sitosterol, and α-terpinene. Enzyme induction was determined by 2 response variables, 1) *in vitro* detoxification enzyme analysis and 2) tolerance to insecticides.

***In vitro* analysis:** The alimentary tract was dissected and gut content removed. The alimentary tract and remaining insect were homogenized in buffer. Differential centrifugation separated sub-cellular fractions into esterases, microsomal oxidases, and glutathione transferases. Increased enzymatic activity was determined by surrogate substrates.

Insecticide Tolerance: Cockroaches fed the augmented diets were anesthetized with CO_2 and topically treated with 1 µl of an acetonic solution of 3 blatticides commonly used in the urban environment, cypermethrin, propoxur, and chlorpyrifos. After 24 h percent mortality was corrected and analyzes with probit. Significant differences between LC_{50}s was failure of 95% CI to overlap. Induction ratio was calculated by dividing the LC_{50} of cockroaches fed the augment diet by the LC_{50} of the cockroaches consuming lab chow.

Results: Sitosterol induced more detoxifying enzymes than the other allelochemicals. Sitosterol elevated specific activity by 1.7, 3.3, 1.2-fold for aldrin epoxidation, methoxyresorufin O-demethylation, and p-nitrophenyl acetate glutathione transerferase, respectively, when compared to the same enzyme assays for cockroaches consuming the lab chow diet. Alpha-naphthol acetate esterases were not affected by ingestion of any allelochemicals tested.

The IRs for cockroaches topically treated with cypermethrin were 1.69-1.81 (Table 1). Similar results were seen when cockroaches were treated with propoxur, i.e., IRs were elevated 1.6-1.7 for cockroaches exposed to the allelochemical diets. The IRs were the same or lower for cockroaches treated with chlorpyrifos.

Plans: This research is continuing.

[1]Department of Entomology and Nematology, University of Florida

Table 1. LC_{50} of Orlando male cockroaches fed 0.2% alleochemicals in lab chow for 4 d. n = 180

Inducer	Treatment	Slope ± SE	LC_{50} (95% CI)[a]	χ^2	IR
Lab diet	Cypermethrin	5.39 ± 0.71	0.016 (0.015-0.020)	2.55	1.00
Barbitol	Cypermethrin	11.11 ± 2.55	0.029 (0.027-0.030)	0.40	1.81
Menthol	Cypermethrin	11.09 ± 1.61	0.027 (0.026-0.029)	1.51	1.69
Sitosterol	Cypermethrin	11.76 ± 1.69	0.028 (0.026-0.030)	1.84	1.75
α-Terpinene	Cypermethrin	17.13 ± 2.54	0.029 (0.027-0.031)	0.05	1.81
Lab diet	Chlorpyrifos	2.38 ± 1.37	0.335 (0.278-0.579)	0.909	1.00
Barbitol	Chlorpyrifos	6.95 ± 2.06	0.336 (0.285-0.589)	0.756	1.00
Menthol	Chlorpyrifos	3.01 ± 1.07	0.319 (0.274-0.493)	1.29	1.00
α-Terpinene	Chlorpyrifos	4.48 ± 0.84	0.231 (0.215-0.257)	3.45	0.69
Lab diet	Propoxur	2.37 ± 0.41	0.238 (0.182-0.289)	2.90	1.00
Barbitol	Propoxur	3.73 ± 0.66	0.375 (0.326-0.458)	2.32	1.58
Menthol	Propoxur	1.08 ± 0.33	0.378 (0.316-0.515)	3.52	1.59
α-Terpinene	Propoxur	3.35 ± 0.97	0.394 (0.294-1.376)	9.09	1.66
Sitoserol	Propoxur	1.25 ± 0.33	0.376 (0.322-0.483)	7.77	1.58

[a]LC_{50} µg/insect

INSECTICIDE RESISTANCE DETECTION IN FIELD POPULATIONS OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA*

S.M. Valles

Objectives: To develop methods capable of identifying potential insecticide failures caused by insecticide resistance in German cockroach field strains.

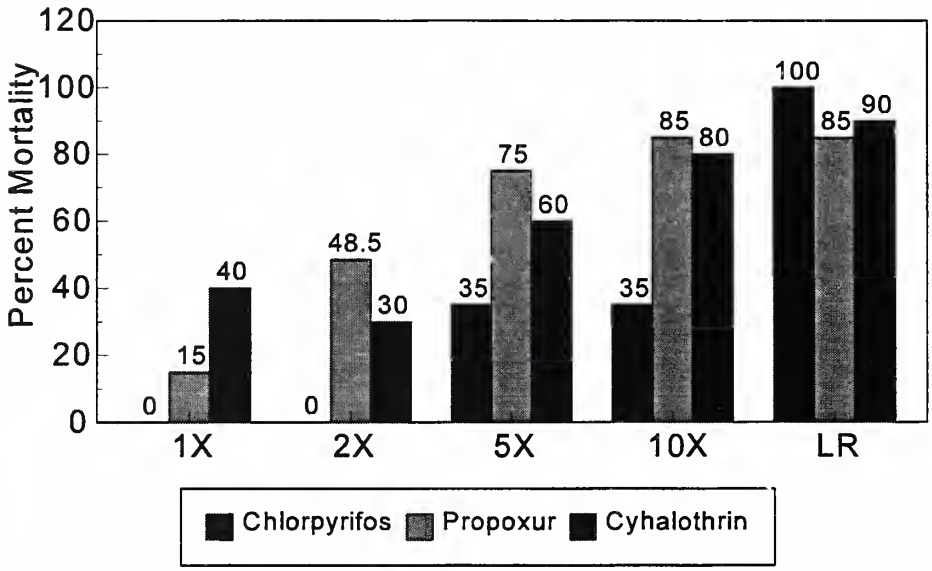
Methods: Insecticide resistance can significantly reduce the ability to control German cockroaches effectively. Past attempts to detect insecticide resistant populations have relied upon common sticky traps impregnated with a known quantity of insecticide. With several classes of insecticides used in separate traps and in the same structure, it was possible to identify insecticide resistant cockroaches by survival after a known period of time on the residue. Unfortunately, problems with glue potentiation of insecticides, unknown time on the residue, differential stage susceptibility, very low trap catches, insecticide repellency, and environmental disposal of used traps precluded the further development of this method.

An alternative method based on residual bioassays was developed. Four laboratory strains of German cockroach were tested against λ-cyhalothrin, propoxur, and chlorpyrifos in jar bioassays. Known quantities of insecticide in acetone were placed into a glass jar (90 cm^2 area). The jar was swirled gently until the acetone evaporated to coat the interior surface. Ten cockroaches were placed onto the insecticide residue for 24 hours. Since stage-dependent insecticide susceptibility has been reported in the German cockroach, small and large nymphs and adult males were used in the bioassays. Six to ten concentrations were used to develop log-dosage mortality curves for 1 susceptible and 3 resistant laboratory strains of German cockroach. With this information, diagnostic insecticide doses were tested against various field strains of German cockroaches. Cockroaches were collected actively with a vacuum-assisted device developed in this lab. Relative insecticide tolerance of the field strains was assessed within the genetic background of the laboratory strains.

Results: Five diagnostic doses (1-, 2-, 5-, and 10-times the LD_{99} of the susceptible lab strain and the labeled field rate for each insecticide) were chosen to evaluate field cockroaches. As of October 1996, fifteen strains have been collected from the field. Of these, four had been evaluated against the three insecticides mentioned. Using the $5XLD_{99}$ value as the demarcation between resistant and "susceptible," 3/4, 4/4, and 2/4 of the strains were resistant to chlorpyrifos, propoxur, and λ-cyhalothrin, respectively. Although this is admittedly a small sample size, it appears that insecticide resistance may be pervasive toward these insecticides. In addition, the detoxification capacity of the field strains is being examined as potential indicators of resistance. Such an anomaly could be exploited by molecular or immunological means to develop a colorimetric method to detect insecticide resistance in field cockroaches.

Plans: This line of research will continue with the intention of ultimately providing a method that can be used in the field to detect resistance.

Level of Insecticide Resistance in a German Cockroach Field Strain



VACUUM-ASSISTED DEVICE FOR COLLECTION OF LIVE GERMAN COCKROACHES FROM THE FIELD

S.M. Valles

Objectives: Develop a device or method capable of actively collecting German cockroaches from the field in sufficient numbers to be used immediately in insecticide resistance detection assays.

Methods: Anticipating the need for collecting sufficient numbers of German cockroaches from the field for use in insecticide resistance detection assays, a vacuum-assisted collection apparatus was designed and built. Traditional jar traps baited with beer and bread are incapable of capturing sufficient numbers of cockroaches for immediate use in research. The vacuum-assisted device allows for active collections to occur, as opposed to passive trapping, that are fast, minimally disruptive to the occupants, and capable collecting large numbers of cockroaches.

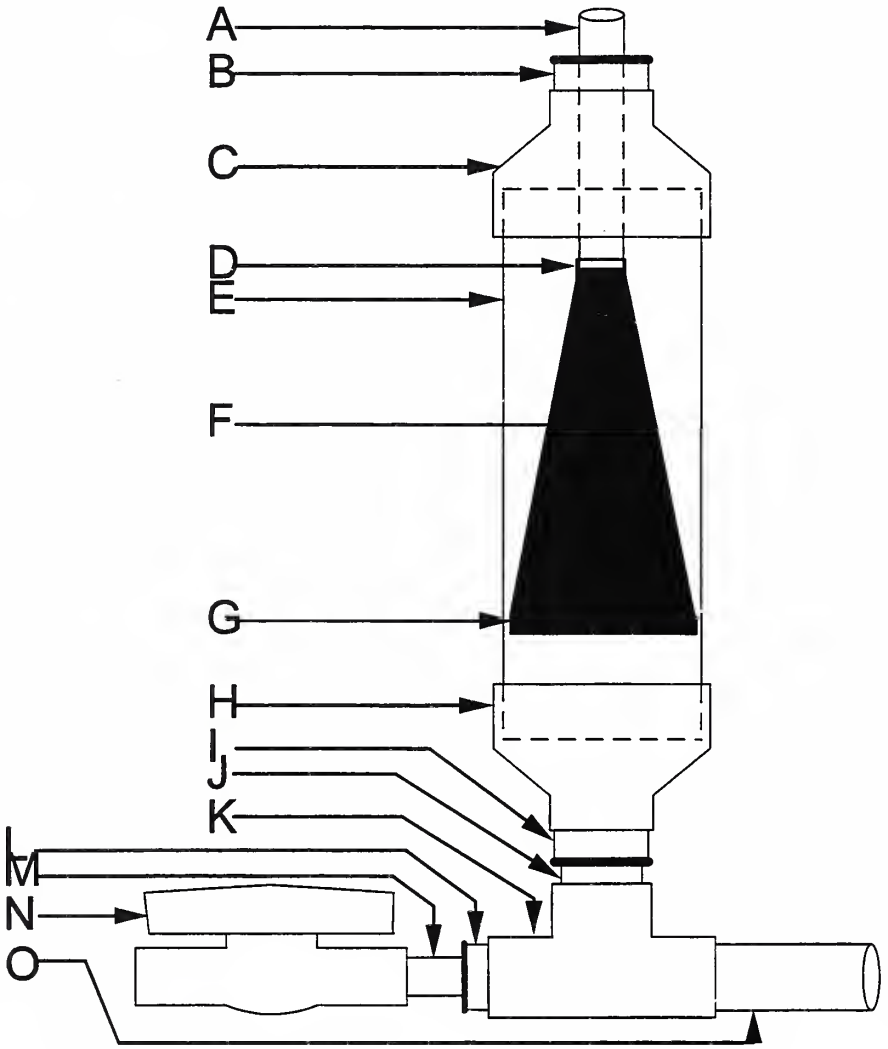
The vacuum-assisted cockroach collector (VARC) is constructed primarily from PVC pipe and related fittings. All components can be purchased from a home center or hardware store for around twenty five dollars (October 1996).

Results: The accompanying figure is a diagrammatic sketch of the VARC. A vacuum source (canister shop vacuum or li'l hummer®) is connected to the vacuum source pipe (O). Cockroaches are sucked into the vacuum inlet by way of a flexible hose attachment. All stages are captured in the cone-shaped 6 mesh fiberglass screen bag. After collecting, the fiberglass bag is detached at pipe "P" and the cockroaches are dumped into a suitable container or placed into a mailing tube for transport.

The VARC is an active collection method providing the ability to catch all developmental stages and in higher quantity compared with passive trapping methods. For example, six beer and bread baited glass jar traps yielded 35 cockroaches in a 24 hour period in a kitchen artificially infested with approximately 500 German cockroaches. Under the same conditions, the VARC caught 175 cockroaches in 5 minutes.

Plans: The VARC has been distributed to several pest control firms for the purpose of collecting field strains typically encountered by these professionals. Research is scheduled to develop a better understanding of insecticide tolerance and resistance in these German cockroach field strains.

Diagrammatic Sketch of the Vacuum-Assisted Cockroach Collector



AN INHIBITOR OF CYTOCHROME P450 MONOOXYGENASES IN GERMAN COCKROACH (DICTYOPTERA: BLATTELLIDAE) MIDGUT CONTENTS

S.M. Valles and S.J. Yu[1]

Objectives: To identify and characterize an endogenous inhibitor of cytochrome P450 monooxygenases found in the German cockroach midgut.

Methods: Current methods of microsome preparation in German cockroaches includes the homogenization of the entire thorax and abdomen in a buffer (typically sodium phosphate, pH ~7.5) containing chemicals intended to protect cytochrome P450 monooxygenases (e.g., phenylmethylsulfonyl fluoride, 1-phenyl-2-thiourea, glycerol, EDTA, and dithiothreitol). Unfortunately, significant inhibition of cytochrome P450 catalyzed reactions often occurs when microsomes are prepared in this manner. The inhibitory factor was isolated to the midgut contents which are liberated upon homogenization of the tissues. Experiments were performed to document the existence, potency and possible mechanism of this midgut content inhibitory factor in the German cockroach, *Blattella germanica* (L.).

Microsomes were prepared from adult and final instar male and female German cockroaches with gut contents *in situ* or removed prior to homogenization and compared with respect to a cytochrome P450-catalyzed reaction (methoxyresorufin (MR) *O*-demethylation). In addition, time and dose dependent effects of the inhibitor on MR *O*-demethylase activity were also evaluated.

Results: Midgut content removal prior to homogenization resulted in a 4- to 10-fold increase in MR *O*-demethylase activity in final instar male and female nymphs, respectively. However, the presence of midgut contents had no significant effect on MR O-demethylase activity in adult males. These stage dependent differences may be related to food consumption differences among the two stages. Since the nymphs are an active feeding stage, the inhibitor may be a digestive enzyme(s) released into the midgut in response to food consumption. Indeed, this hypothesis is indirectly supported by the fact that within the final instar, food consumption and cytochrome P450 inhibition are correlated (when microsomes are prepared from whole insects with the gut contents *in situ*). Dose and time dependent inhibition also occurred in both stages when gut contents were analyzed *in vitro*.

Gut content removal significantly improved cytochrome P450 activity. If the gut contents are not removed, erroneous conclusions may be drawn due to enzyme inhibition.

Plans: This work has been completed.

[1]Department of Entomology & Nematology, University of Florida

INTERCONTINENTAL DIFFERENCES IN THE ABUNDANCE OF FIRE ANTS: AN ESCAPE FROM NATURAL ENEMIES?

S.D. Porter, D.F. Williams, R.S. Patterson, and H.G. Fowler[1]

Objectives: To measure intercontinental differences in fire ant abundance and to determine if the unusually high populations of fire ants in the United States can be explained by factors such as climate, habitat, geography, seasonal variability, differences in population structure, or escape from natural enemies.

Methods: To compare intercontinental fire ant densities, we selected 13 areas in South America and another 12 areas in North America. Sample areas were distributed across a broad range of climatic conditions. In each area, we measured fire ant densities at 5 preselected roadside sites. At each site, we also measured foraging activity, checked for polygyne colonies, and recorded environmental data. In most areas, we also measured fire ant densities in lawns and grazing land.

Results: Fire ant populations along roadsides in North America were 4-7 times higher than fire ant populations in South America. Similar intercontinental differences were found in lawns and on grazing lands. These intercontinental differences were not associated with sampling conditions, seasonal variability, habitat differences, or the frequency of polygyny. Although several correlations were found with long-term weather conditions, careful inspection of the data suggests that these correlations were probably more coincidental than causal. Bait tests showed that competition with other ants was more important in South America; however, we were not able to determine whether this was a major cause of intercontinental differences or largely a consequence of other factors such as the numerous pathogens and parasites that are found in South America. Because this study was correlational, we were unable to determine the cause(s) of the large intercontinental difference in fire ant abundance that we observed. However, we were able to largely exclude a number of possible explanations for the differences, including sampling, season, polygyny, climate, and aspects of habitat. By a process of elimination, escape from natural enemies remains among the most likely explanations for the unusually high densities of fire ants found in North America.

Plans: This work has been completed.

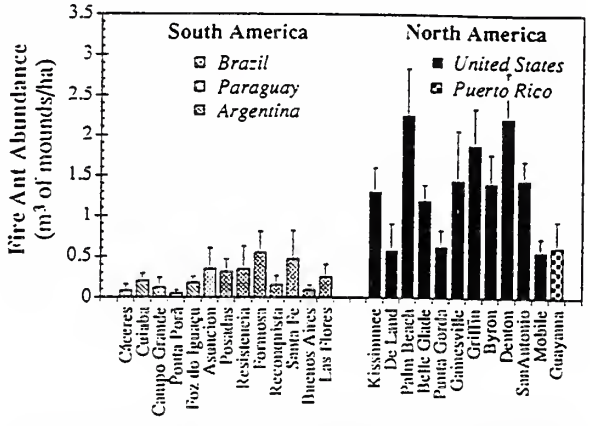


Fig. 1. Fire ant abundance in cubic meters of mound volume per hectare for 13 roadside areas in South America and 12 roadside areas in North America. Each bar indicates the mean and standard error for 1 area; each area contained 5 sample sites.

[1]Departamento de Ecologia, Instituto de Biociencias, Universidade Estadual Paulista, 13500, Rio Claro, SP, Brazil

FIRE ANT ALATE EXCITANT PHEROMONE

R.K. Vander Meer and L.E. Tennant de Alonso

Objectives: To determine the glandular source of fire ant alate sexual, male and female, mating flight excitant pheromones.

Methods: Laboratory reared fire ant colonies were induced to fly in the laboratory using standard procedures. "Mating flight activated" (MFA) alates were collected just before take off and were tested immediately. The bioassay units consisted of worker groups in individual plastic trays. The bioassay was conducted blind to the observer and assistant. Test samples consisted of 3 mL of air drawn by a syringe from a control or sample vial, then 1 mL of air from the syringe was released over the workers. Worker reactions were recorded. Samples were placed in a 7-mL glass scintillation vials and included live alates, individual head, thorax, or gaster samples, mineral oil solutions of glandular products from mandibular glands, post-pharyngeal glands, and poison sacs excised in water under a binocular dissecting microscope from alates that had initiated pre-flight activity. For each test, five ul of solution (1.6 alate equivalents) was applied to a thin strip of filter paper and placed in a sample vial. Data were analyzed using the McNemar test for significance of changes.

Results: Air from vials containing live mating flight activated female and male alates elicited highly significant excited reactions in *S. invicta* worker groups. Crushed heads of all alate categories stimulated highly significant excitement in workers. Results for crushed female thoraces were variable while crushed male thoraces elicited excitement at a lower level of significance than crushed heads ($p<0.05$ vs. <0.001, respectively). It is not clear yet whether the thorax results are due to contamination by substances released from the mandibular glands during separation of the body parts or if a glandular source from the thorax is responsible. Crushed gasters of all female alate categories but not male gasters produced some level of excitement in the workers, as did crushed poison gland solutions from winter MFA females. Mineral oil extracts of mandibular glands from female and male alates produced significant excitement in workers. Crushed heads without mandibular glands did not elicit excitement in workers ($p>0.05$) while heads with intact glands tested at the same time elicited excitement at a highly significant level ($p<0.001$). No excitement was elicited by post-pharyngeal gland solutions from any of the alate categories. Our results show that the mandibular glands are a source of an excitant pheromone in both female and male *S. invicta* alates. In our bioassay, *S. invicta* workers consistently reacted with rapid movement and frantic running when exposed to live alates, crushed heads, and mandibular gland solutions.

Plans: This part of the study has been completed. Research on the isolation and identification of the excitant pheromones is currently being conducted.

CONTROL OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) WITH TEFLUBENZURON

D.F. Williams

Objectives: To develop and evaluate new toxicants and insect growth regulators for the control of urban pest ants.

Methods: Laboratory and field tests were conducted with the insect growth regulator, teflubenzuron. For laboratory tests, teflubenzuron (10% emulsifiable concentrate) was combined with once-refined soybean oil (ORSBO) to produce baits containing 0.1% and 0.5% active ingredient (wt/wt). In each test, three fire ant colonies were exposed to 0.5 mL of each bait concentration. The 0.1% solution (0.5 mg per colony AI) and the 0.5% solution (2.5 mg per colony AI) were tested against colonies with 20-25 mL brood and 20,000-40000 workers, and 30-35 mL brood and 50,000-70,000 workers, respectively. Three colonies were exposed to 0.5 mL of neat soybean oil and served as non-treated controls. The test colonies were allowed ad libitum feeding on the oil solutions which were offered in micropipets. The colonies were returned to normal diet (Banks et al. 1981) 24 h after treatment and maintained in the laboratory at 27±2°C. Monthly observations (including numbers of workers, reproductives, and amount of brood) were made until the colonies died, returned to their normal pretreatment index level, or for one year, whichever occurred first. Effectiveness of the treatments was based on comparison of the before and after treatment size index of each colony. In field tests, pregel defatted corn grit baits containing 0.01125, 0.0225, or 0.045% teflubenzuron were prepared as follows. Technical teflubenzuron (97.5%) was dissolved in dimethyl formamide (0.5-1.5% by weight of oil in the formulation) and the solution was incorporated into warm (20-25°C) ORSBO. The oil solution was slowly poured over the corn grits as they were stirred in a large food mixer. Stirring continued for about 10 minutes to insure thorough mixing of the oil and grits. Each bait was broadcast with a tractor-mounted granular applicator at a rate of 1.12 kg/ha on 0.2- ha plots with an average of 12 mounds per hectare in nongrazed permanent pasture in Union County, Florida. Three plots were treated with each teflubenzuron concentration; three plots were treated with Logic (fenoxycarb, Ciba-Geigy, Greensboro, NC) at a rate of 1.12 kg/ha as a standard, and three plots were left untreated as a control. Efficacy of the treatments was evaluated by comparison of the before and after(6, 13 and 17 weeks) treatment population indices using standard methods established for determination of population indices of *S. invicta*.

Results: Teflubenzuron baits were very active against laboratory colonies of the red imported fire ant, *Solenopsis invicta* Buren. Worker brood production ceased soon after treatment and by four weeks posttreatment, most colonies were devoid of brood. Worker ants did not exhibit any direct effects from treatment with teflubenzuron. As is typical with most insect growth regulators, colony mortality was slow and dependent on old-age attrition of the worker ants. A few (<25) female alates were produced in one of the laboratory tests at 12 weeks posttreatment. The teflubenzuron baits reduced field colonies of *S. invicta*by 75-79% within 6 weeks after treatment, 83-86% within 13 weeks, and 77-91% within 17 weeks. At 17 weeks posttreatment, the presence of worker brood in the plots treated with the lower rates, 0.1125% and 0.0225%, gave evidence of recovery of some colonies. However, the results of the field tests indicate that teflubenzuron has excellent potential for control of field populations of *S. invicta*.

Plans: This work has been completed.

IMPACT OF RED IMPORTED FIRE ANT POPULATIONS ON SELECTED ENDANGERED WILDLIFE IN THE FLORIDA KEYS[1]

D.P. Wojcik, C.R. Allen[2], and E.A. Forys

Objectives: To study red imported fire ants populations in selected habitats and the effects of fire ants predation on lizard, and turtle eggs and hatchlings, Stock Island Tree Snails, and Salt marsh rabbits.

Methods: Study locations were selected based on known or potential distribution of several of the endangered species. Bait transects, usually 10 sites each with a meat & a honey bait, were located in the available ecotones: berm, transitional, etc. Where appropriate, arboreal baits and test-tube pitfalls were also used. The baits were left in position for one hour, collected in plastic cups with lids, frozen, and the ants preserved in alcohol. The ants were identified in the laboratory. Where arboreal baits and test-tube pitfalls were used, they were placed at the same approximate locations as the ground baits. Arboreal baits were handled in a like manner to the ground baits. Test-tube pitfalls, containing 50% propylene glycol, were placed in the ground and left in place for 4 days. The test-tubes were removed, corked, and returned to the laboratory for separation and identification.

Results: The 3,000 samples from 120+ locations have been collected and are being processed. An initial identification based on presence or absence of fire ants has been completed and the information sent to cooperators to select sites for paired insecticide treatments.

Plans: The samples are being used to select sites for paired insecticide treatment tests, scheduled for March-May 1997 in the Florida keys in selected habitats to demonstrate the need for Red imported fire ants control. Predation tests will be conducted at the Gainesville laboratory using surrogates to demonstrate whether Red imported fire ants feds on endangered tree snails, caterpillars, or hatchling turtles. This information will be used by the State of Florida in the selection of suitable habitats for the reintroduction of the Stock Island Tree Snail.

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[1] This research is supported in part by a grant received from the Florida Game & Fresh Water Fish Commission
[2] Florida Cooperative Fish & Wildlife Cooperative Research Unit, Department of Wildlife Ecology & Conservation, University of Florida

CRIS - 6615-32000-029-00D

REARING THE DECAPITATING FLY *PSEUDACTEON TRICUSPIS* IN IMPORTED FIRE ANTS FROM THE UNITED STATES

S.D. Porter, D.F. Williams, and R.S. Patterson

Objectives: To determine if *Pseudacteon* flies which attack fire ants in South America would oviposit and develop successfully on imported fire ants in the United States. Our second objective was to develop a way of rearing these flies under laboratory conditions to facilitate future inoculative releases in the United States.

Methods: About 80 *S. richteri* workers were collected during the first week of April 1995 after having been attacked by ovipositing *Pseudacteon tricuspis* flies in Las Flores, Buenos Aires Provence, Argentina. We also collected 11 female and 26 male *P. tricuspis* flies. Adult flies and the parasitized workers were hand-carried to Gainesville, FL, on the morning of 7 April. All work in Gainesville was conducted in quarantine facilities operated by the Florida Department of Agriculture. We released the flies into 3 vented flight boxes with medium and large fire ant workers. The boxes were placed near a window under fluorescent room lighting. Workers attacked by flies were combined in groups of 4-10 with several dozen minor worker adults and brood from their mother colony. These fragment colonies were placed in small foraging trays with test tube nests. All test ants were provided daily with frozen crickets and 1 M sugar water.

Results: We found that this fly developed successfully on *Solenopsis invicta* workers from Florida and hybrid *Solenopsis* fire ants from Mississippi. It also was reared on *S. richteri* and *S. invicta* fire ants from Argentina. This fly, like its congener *Pseudacteon litoralis*, had the peculiar habit of decapitating its living host and using the ant's empty head capsule as its pupal case. Just prior to pupation, *P. tricuspis* apparently releases an enzyme or hormone which causes the degeneration of the cuticular membranes that connect the ant head and the 1st pair of legs to the thorax. The maggots consumed all of the tissue in the head of its host, then cut away the maxillo-labial plate and generally one or both mandibles. Upon pupation, the 1st 3 segments of the maggot compressed and sclerotized to form a specialized cap that precisely filled the oral cavity of the ant head. We found that *P. tricuspis* required 20 ± 5 d to hatch and complete larval development, and another 19 ± 2 d to complete the pupal stage for a total developmental time of 39 ± 4 d (n = 12, ≈24°C). We were able to rear this parasite through 1 complete generation, including mating and oviposition under laboratory conditions. This accomplishment was important because it suggested that mass rearing of this parasite for inoculative release might be possible with improved rearing techniques.

Plans: We have recently developed techniques for rearing large numbers of *Pseudacteon* flies in the laboratory and have completed a series of host specificity tests that indicate that these flies pose no threat to any organism in the United States except fire ants. We will apply for field release permission shortly and hope to begin field release trials sometime next spring.

EPIZOOTIC OF A MICROBIAL PATHOGEN IN THE IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*, IN THE UNITED STATES

D.F. Williams, G.J. Knue, and J.J. Becnel

Objectives: To develop biological control agents for imported fire ants in the United States.

Methods: When we collect fire ant colonies from field sites for use in our studies, we routinely survey 100 workers from each colony for pathogens that might be useful as biological control agents. It was during this type of survey of 10 polygyne field-collected colonies that we discovered a microsporidium in workers of *Solenopsis invicta*. The spores appeared similar to those of the microsporidium, *Thelohania solenopsae*. This is the first time a microbial pathogen has been found in *S. invicta* in the U.S. Following the discovery of infected workers, 30 colonies were excavated from this area and taken to the laboratory where the ants were separated from the soil by flotation. To detect the percent of colonies infected, a sample containing 50-100 workers was ground in a glass tissue grinder with ca. 1 mL of water; one drop of the aqueous extract was examined by phase-contrast microscopy (400x) for the presence of spores. Numbers of individuals of the different castes and stages were randomly sampled from each of the infected colonies. Individual worker ants and queens were examined for the presence of spores (meiospores and free spores) of the microsporidium by smearing the contents of their gasters on a microscope slide, adding a drop of water, placing a cover slip over the mixture and surveying under the microscope.

Results: A total of 379 colonies was excavated in north central Florida and 86 (23%) of these were infected (Table 1). Workers had the highest infection rate (72%) followed by larvae (54%) and queens (31%). We have also examined colonies from Hurley, MS (3 of 8 colonies were infected), Gulfport, MS (1 of 8 colonies was infected) and Thorndale, TX (5 of 7 colonies were infected). In addition, we have examined colonies of the following other ant species: *S. geminata* Fabricius (15), *Dorymyrmex bureni* (Trager) (9), *Pheidole metallescens* Emery (1), *Pheidole moerens* Wheeler (1), *Camponotus floridanus* (Buckley) (1), *Trachymyrmex septentrionalis* McCook (1), and *Brachymyrmex depilis* Emery (1) and all were negative for the microsporidium. Meiospores (n=25) had a length and width (mean and +SE) of 3.722 ± 0.049 and 2.013 ± 0.023 microns, respectively. Free spores were rare (<1%) and were not measured. It is interesting to note that more than expected abnormal meiospores were observed. Vegetative stages of the microsporidium were found in the larvae of imported fire ants by microscopic examination (400 and 1000x) of Giemsa-stained smears of 4th instar larvae. Spores were not present in larvae or pupae. At the present time, only polygynous *S. invicta* colonies have been found to be infected. One explanation may be that this microsporidium is directly transmissible and because polygynous colonies, unlike monogyne colonies, are not aggressive towards each other, workers and brood move frequently between colonies. Thus, infected colonies could easily infect healthy colonies in a polygynous fire ant population when infected workers and brood are moved to a healthy colony. The detrimental effects on *S. invicta* field colonies is not known at present but of the original 30 field-collected colonies that were infected and returned to the laboratory, all are completely without brood and have only a few workers and queens remaining. This is in contrast to healthy field-collected colonies that have not only survived in our laboratory for several years without loss of brood but have increased in size. The origin of the microsporidian pathogen responsible for this epizootic in the U.S. is unknown.

Plans: Transmission, morphological, and molecular studies are underway to determine if this microsporidian pathogen is conspecific with *Thelohania solenopsae* with origins in South America or represents a new species from North America.

TABLE 1

Number (%) of Individuals of *Solenopsis invicta* Infected with an unknown Microsporidium

Castes or stages	Number of individuals examined	Number of (%) individuals infected
Larvae I-IV	78	42 (54)
Workers	60	43 (72)
Queens	16	5 (31)
Colonies	379	86 (23)

MOSQUITO

AND

FLY

CRIS - 6615-32000-031-00D -- Repellent Systems and Control Strategies for Mosquito/Vectors of Medical and Veterinary Importance

CRIS - 6615-32000-032-00D -- Biological Control and Integrated Management of Bloodsucking and Nuisance Flies of Med/Ag/Vet Importance

CRIS - 6615-32000-031-00D

CAGE SIZE, MOSQUITO DENSITY, AND BITING RATE EFFECTS IN LABORATORY TESTS OF REPELLENT

D.R. BARNARD, K.H. POSEY, & D. SMITH

Objective: In this study we sought to determine the relationship between cage size, mosquito density, and the protection period provided by the repellent, deet.

Methods: Three cage sizes: large (50 × 50 × 50 cm), medium (46 × 38 × 37), and small (30 × 30 × 30 cm) and three mosquito densities were used: low (1 female/640 cm^3 of cage space), medium (1 female/128 cm^3 of cage space), and high (1 female/ 49 cm^3 of cage space). The repellency of deet to 7-8 day-old nulliparous *Ae. aegypti* and *An. quadrimaculatus* was determined for each treatment combination using a 3 × 3 factorial design. Repellent treatment consisted of application of 1 ml of a 25% ethanolic deet solution to the forearm of a volunteer. In each test the volunteer placed their arm into a cage of mosquitoes for 3 min and observed the numbers of mosquitoes that landed and attempted to feed. Observations were repeated at 90-min intervals until a confirmed mosquito bite was recorded.

Results: The repellent protection time (RPT) of deet against *Ae. aegypti* ranged from 4.5 to 6.5 h and was shorter (5.0 ± 0.8 h) in large cages compared with medium (6.2 ± 0.9 h) and small cages (6.2 ± 0.8 h). In *An. quadrimaculatus*, protection times ranged from 1.5 to 8.0 h, were shortest in small (3.7 ± 2.3 h) and large cages (2.2 ± 1.1 h) at medium (3.7 ± 2.3 h) and high mosquito densities (2.5 ± 1.7 h), and longest in medium cages (6.2 ± 2.6 h) at low mosquito densities (5.8 ± 2.8 h). Using a standard dose of deet, the shortest repellent protection times were observed in large cages with high densities of mosquitoes. The longest protection times were observed in medium cages with low mosquito densities.

We analyzed RPT for both *Ae. aegypti* and *An. quadrimaculatus* in relation to cage size and mosquito density conditions. The results showed that expected repellent protection times for both species changed in a straight line manner when measured against changes in mosquito density and in a curved line manner when measured against changes in cage size (Figure 1).

Mosquito density and biting rate also were found to be positively correlated, whereas biting rate and repellent protection times were negatively correlated . Regression analyses of these data showed that a significant portion of protection time responses in *Ae. aegypti* and *An. quadrimaculatus* could be explained by mosquito biting rate. The RPT for deet against *Ae. aegypti*, when estimated on this basis, ranged from 4.6 h (at 62 bites per half min) to 6.2 h (at 3-9 bites per half min) and for *An. quadrimaculatus*, from 1.8 h (at 50-60 bites per half min) to 6.5 h (at 2 bites per half min) (Figure 2).

In *Ae. aegypti*, increases in biting pressure have a relatively minor effect on RPT. Using the lower 95% confidence limit for estimates of RPT as a guide (Figure 2), the minimum time to first bite, over the observed range of biting rates, is 2.5 hours. In contrast, in *An. quadrimaculatus,* increases in biting pressure have a pronounced effect on protection time Based on the lower 95% confidence limit for estimates of RPT for this mosquito (Figure 2), the expected minimum protection time in 95 out of 100 repellent bioassays is 1 h when the biting rate is 6 or fewer bites per half min. The minimum expected RPT at biting rates $\geq$ 15 per half min is 0.

Plans: This phase of the study has been completed and a manuscript entitled 'Cage size, mosquito density, and biting rate effects in laboratory tests of repellent' has been submitted for publication.

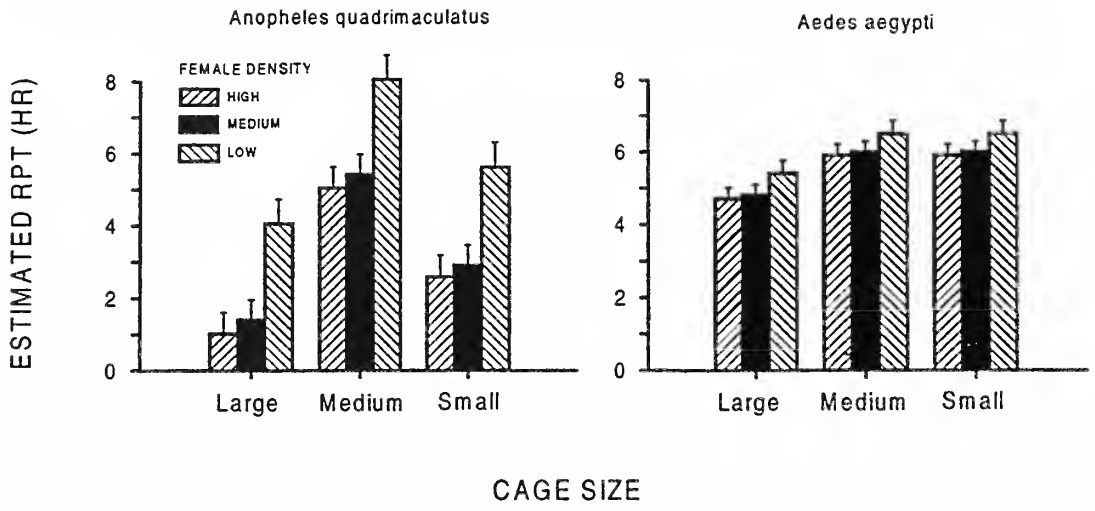


Figure 1

Estimated repellent (25% deet) protection time in relation to the cage size and the density of female mosquitoes used in repellent bioassays.

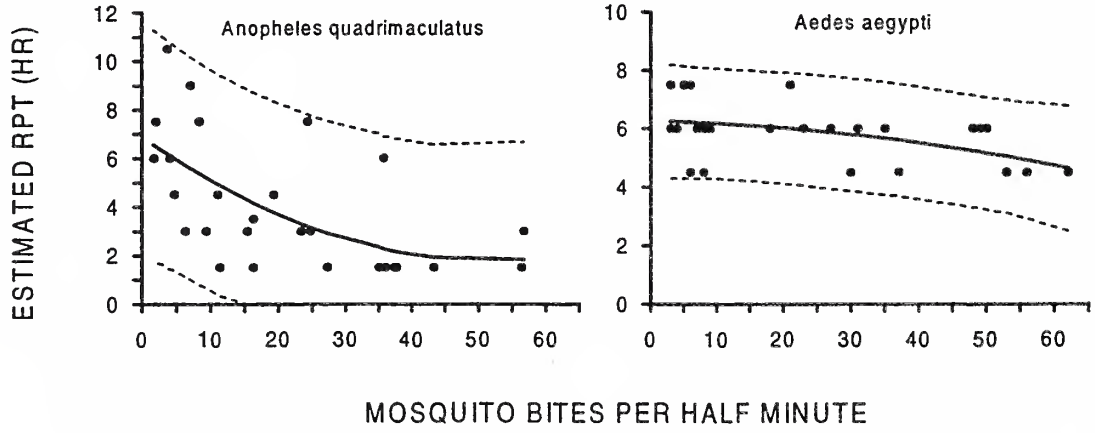


Figure 2

Observed (●) and estimated (▬) repellent protection times in relation to mosquito biting rate. Dotted lines represent upper and lower 95% confidence limits for estimated repellent protection time.

MOSQUITO CROP SUGAR HYDROLYSIS AND IDENTIFICATION

D.A. Burkett[1], D.A. Carlson and D.L. Kline

Objectives: To determine if hydrolysis of natural mono-, di- and trisaccharides occurs in the crops of *Aedes albopictus* and to evaluate the common dietary sugar composition in the crops of wild *Ae. albopictus*, *Culex nigripalpus*, *Anopheles quadrimaculatus*, and *Culiseta melanura*. To determine the percentage of these populations that obtain their sugar from honeydew.

Methods: Time series analyses were conducted to determine if sugar hydrolysis occurs in the crop of mosquitoes. Two day-old *Ae. albopictus* were starved and fed 10 percent solutions of glucose (monosaccharide), sucrose (disaccharide) and melezitose (trisaccharide; honeydew). Male and females were sacrificed at 2, 4, 8, and 20 hours after feeding. Crop contents were removed, derivatized with Tri-Sil Z® and analyzed against standards using an HP 5980 gas chromatograph. For the second experiment, wild *Ae. albopictus*, *Culex nigripalpus*, *Anopheles quadrimaculatus*, and *Culiseta melanura* were collected in the morning from tree holes and other natural resting sites and analyzed as before.

Results: Preliminary results show that 20-30 percent of the ingested melezitose is hydrolyzed into fructose and glucose within 20 hours. Sucrose is completely hydrolyzed into glucose and fructose after 2 hours, whereas glucose remains largely unchanged after 20 hours. No difference in the hydrolysis of sugars was found between males and females. A significant percentage of wild mosquitoes contained common "plant sugars"in their crops. Of these, about 10-30 percent contained melezitose, which suggested that mosquitoes ingest honeydew as a primary sugar source.

Plans: This work will be part of a Ph.D. dissertation. Many more samples of wild mosquitoes will need to be sampled and analyzed to determine the percentage and importance of honeydew as a source of sugar for wild mosquitoes.

[1]Captain, US Air Force, Graduate Student, Dept. of Entomology and Nematology, University of Florida

EVALUATION OF COLORED LIGHT EMITTING DIODES AS ATTRACTANTS FOR CENTRAL FLORIDA WOODLAND MOSQUITOES

D.A. Burkett[1], D.L. Kline, J.F. Butler[2]

Objectives: To develop improved trapping efficiency for various host seeking mosquito species in modified CDC traps using low cost, high intensity colored light emitting diodes (LED).

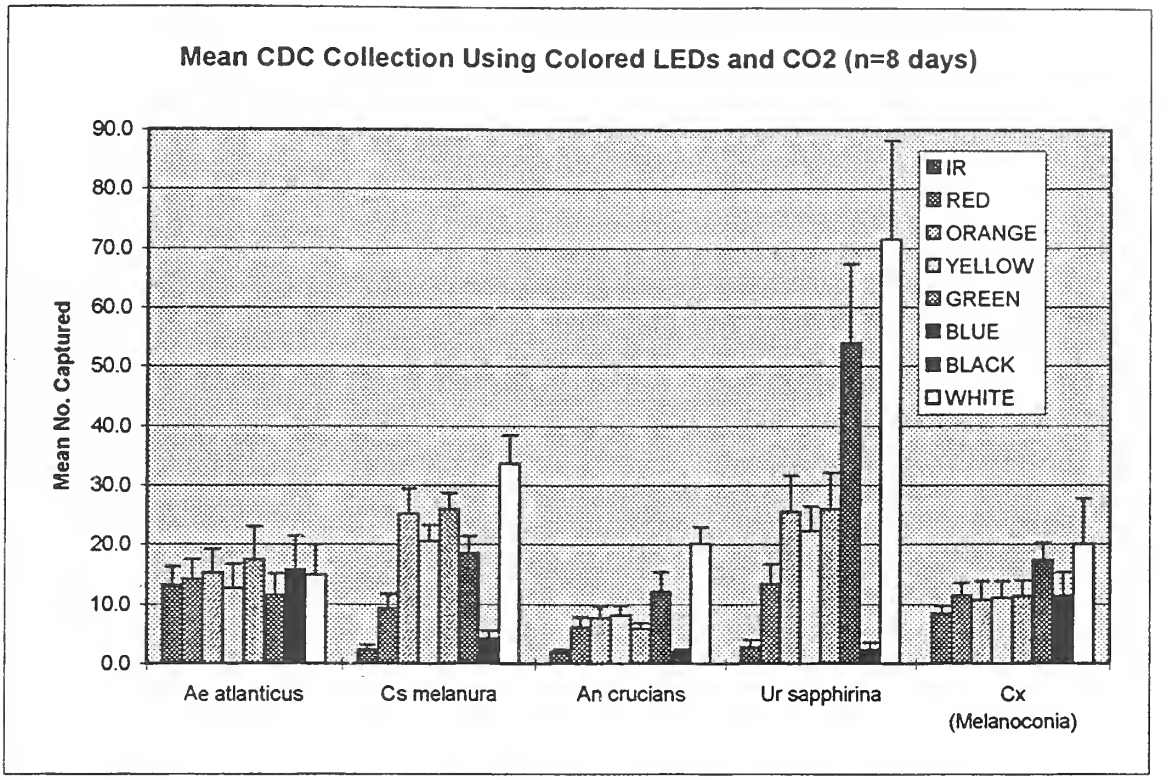
Methods: The attraction of host-seeking mosquitoes to transmitted light from 'ultra-bright' colored light emitting diodes (LED) (100 nm bandwidth) was evaluated by comparison of capture numbers from CDC traps baited with carbon dioxide (200 ml/min). Traps with either colored LEDs or control lights were arranged in a 8 X 8 Latin square design in a central Florida woodland/ cypress swamp location and checked daily during August 1996. Five different colored "super bright" LED's were compared with infrared, no light, and the standard GE, 6.3 V miniature lamp incandescent bulb. Infrared (940 ±50 nm, 22°), red (630 ± 50 nm, 1600 mcd, 22°); orange (605 ± 50 nm, 2000 mcd, 22°); yellow (587 ± 50 nm, 2300 mcd, 22°) and yellow green (567 ± 50 nm, 2400 mcd, 8°) were the LED's tested. Standard John Hock® CDC-type model 512 traps were modified by replacing the standard bulbs with the LED's. After each trap night, the mosquitoes were sorted, counted and identified to species.

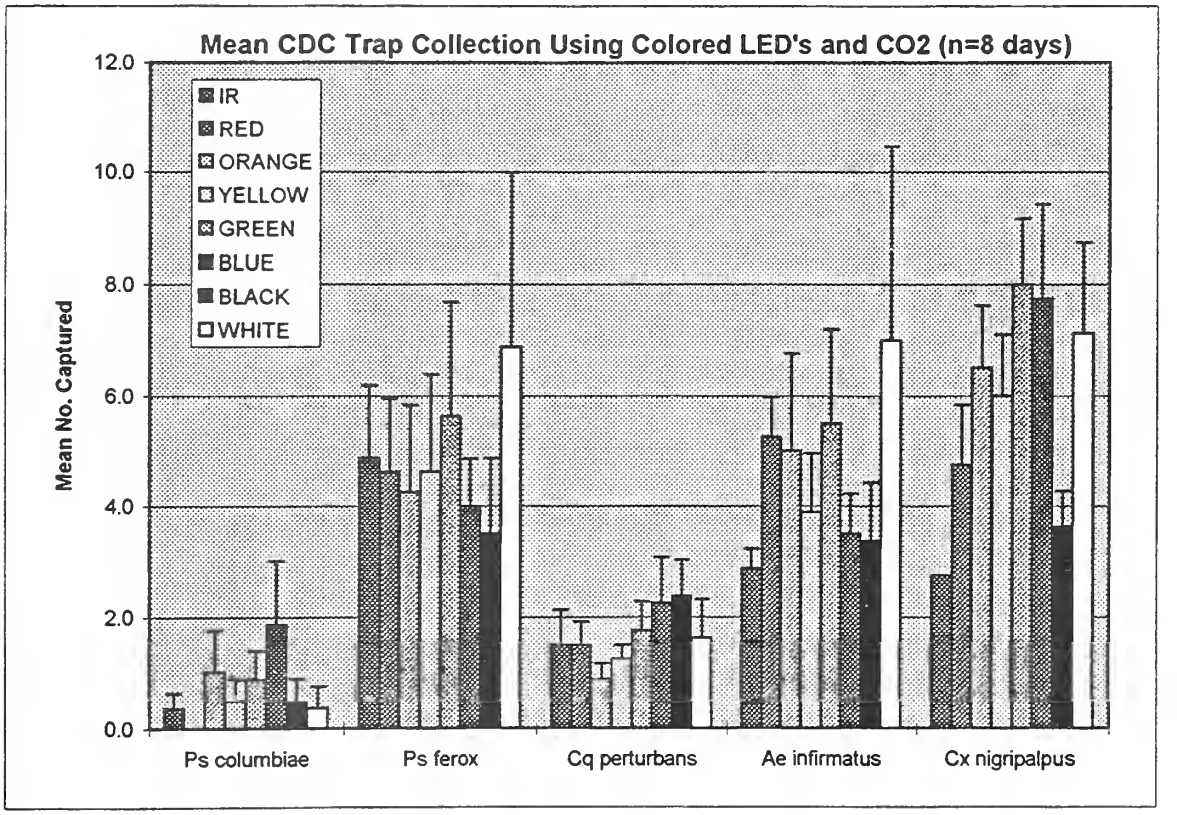
Results: Overall capture of mosquitoes was greatest with the standard white broad spectrum incandescent, followed by blue (450 nm), green (567 nm), orange (605 nm), yellow (587 nm), red (630 nm), black (no light), and infrared (940 nm), respectively. When collections were classified by mosquito species, clear preferences were seen between species.

Significantly more *Anopheles crucians*, *Uranotaenia sapphirina* and *Culex (Melanoconion) spp.* were captured in traps with standard white lights or blue LEDs. The greatest numbers of *Psorophora columbiae* were collected in traps with blue LEDs. Traps with white light, green or orange LEDs captured the most *Culiseta melanura* and those with white light or green LEDs captured the most *Ps ferox*. *Culex nigripalpus* were significantly more abundant in traps with white light, green or blue LEDs and *Coquillettidia perturbans* were most abundant in traps with blue LEDs or no light (black). Traps with white light, green, red or orange LEDs captured the most *Aedes infirmatus*. There was no difference in abundance of *Aedes atlanticus/tormentor* between traps. These results have potential for use in population dynamics studies or for the development of species specific traps. LED's run on significantly lower amounts of energy (ca. 1 milliamp/8 hours) than incandescent bulbs resulting in substantial savings in battery life and expense.

Plans: This work will be part of aPh.D. dissertation.

[1]Captain, US Air Force, Graduate Student, Dept. of Entomology and Nematology, University of Florida
[2]Dept. of Entomology and Nematology, University of Florida


Mean CDC Collection Using Colored LEDs and CO2 (n=8 days)
Mean No. Captured
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
IR
RED
ORANGE
YELLOW
GREEN
BLUE
BLACK
WHITE
Ae atlanticus
Cs melanura
An crucians
Ur sapphirina
Cx (Melanoconia)


Mean CDC Trap Collection Using Colored LED's and CO2 (n=8 days)
Mean No. Captured
12.0
10.0
8.0
6.0
4.0
2.0
0.0
IR
RED
ORANGE
YELLOW
GREEN
BLUE
BLACK
WHITE
Ps columbiae
Ps ferox
Cq perturbans
Ae infirmatus
Cx nigripalpus

DEVELOPMENT OF INNOVATIVE MOSQUITO CONTROL UTILIZING ATTRACTANTS

D.L. Kline and M.O. Mann

Objective: To develop control technology that utilizes attractant-baited traps/targets for mosquitoes from bay and cypress swamps.

Methods: Field studies were conducted with Model 512 CDC-type traps to determine the responses of mosquitoes associated with bay and cypress swamps to various combinations of chemical attractants and/or light. In the first study, we determined the responses of mosquitoes to traps baited with CO_2 (200 ml/min without light), traps with light only, traps with CO_2 (200 ml/min) and light. In a second study, five release rates of CO_2 (2, 20, 100, 200 and 2000 ml/min) were compared in a 5 x 5 Latin square design, utilizing five trap sites each separated by ca. 50 meters. No light source was used in traps in this experiment. In a third experiment, a 5 x 5 Latin square design was used to assess the responses of mosquitoes to unlighted Model 512 traps baited with butanone, butanone + CO_2, octenol, octenol + CO_2 and CO_2 alone. All CO_2 treatments in these tests were at the 200 ml/min release rate. The average octenol release rate was 2.2 mg/hr. The average butanone release rate was 0.97 ml per hour.

Results: The use of CO_2 resulted in a response by all of the species studied. The pattern of response to increasing CO_2 levels varied from species to species but collection size generally increased as CO_2 release rate increased. In the CO_2 and light trap studies the general pattern for collection size was: CO_2 + light> CO_2 alone> light alone. The combination CO_2 + octenol resulted in a synergistic response in all species except Culiseta melanura, Culex nigripalpus and Cx. restuans. Only two species (Aedes atlanticus and Ae. canadensis) responded in relatively large numbers to octenol alone. Octenol at the release rate tested tends to repel Cs. melanura. Butanone at the release rate tested appeared to be repellent for most species.

Plans: This phase of the study has been completed and a manuscript titled "Evaluation of butanone, carbon dioxide and 1-octen-3-ol as attractants for mosquitoes associated with north central Florida bay and cypress swamps" has been submitted for publication.

DIEL PATTERNS OF PUPATION, EMERGENCE, AND OVIPOSITION OF *Aedes albopictus* IN THE LABORATORY

R. XUE and D.R. BARNARD

Objective: To observe, record, and analyze the daily patterns of pupation, emergence, and oviposition of *Ae. albopictus* in the laboratory.

Methods: Mosquitoes were progeny of recently (1995) colonized adult *Ae. albopictus* collected at Gainesville, FL. Large and small individuals were reared and maintained separately at 27°C and 14:10 [L:D] with photophase: 0600 to 2000 h. Pupation: Late, non-feeding 4th instars were transferred in groups of 70 large or 50 small individuals to new rearing pans containing 1 liter of well water. Following the appearance of the first pupa in the new pans, and at 2 h intervals thereafter, pupae were removed from each pan and their numbers recorded. Emergence: Groups of 100 large or 100 small pupae were placed in 100 ml of well water in separate plastic cups covered with nylon mesh. Once emergence began, new adults were counted and removed from each cup at 2 h intervals. Oviposition: Groups of 5 day-old large and small nulliparous females were blood fed on chicken. Three-hundred blood fed mosquitoes were collected from each group and evenly distributed among 6 cages at the rate of 100 large or 100 small females per cage. Sugar water was available at all times. The oviposition substrate was a strip of white filter paper placed around the inside vertical surface of a black polystyrene cup containing 250 ml of well water. When oviposition started, and at 2 h intervals thereafter, the oviposition cup, paper strip, and water were removed from each cage and replaced with a new cup, strip, and water. This procedure was repeated until oviposition stopped. Paper strips were air-dried and the numbers of eggs on each strip determined by visual inspection. For females in the second gonotrophic cycle (GC2), the procedure used to observe oviposition in females in the first gonotrophic cycle (GC1) was repeated except that parous females were blood fed when 13 days old.

A completely randomized design with 3 replications was used in each study. The experimental unit in pupation and emergence studies was a cohort (i.e., rearing pan) of larvae or pupae; for oviposition, the experimental unit was a cage of 100 blood fed mosquitoes. Percentage responses were subjected to analysis of variance after arcsin transformation. Tukey's HSD test (P= 0.05) was used for means separation.

Results: There was no daily pattern of pupation in *Ae. albopictus*; however, diel patterns of emergence and oviposition in this mosquito were influenced by body size. Emergence rate was highest in large-bodied male mosquitoes at 1600 h and in small males at 1000 h but was lowest in large and small males, respectively, at 2400 h and 0200 h. Peak emergence of females was at 1600 h, regardless of body size; lowest emergence was at 0400 h. Half of all ovipositions by large females in GC1 were at 2000 to 2200 h but at 1800 to 2000 h in GC2. Oviposition by small females in GC1 and GC2 was highest at 1800 h and 1600 h, respectively, and lowest at 0400 h. Half of all ovipositions in small females were at 1600 to 1800 h (Fig. 1).

Plans: This phase of the study has been completed and a manuscript entitled 'Diel patterns of pupation, emergence, and oviposition of *Aedes albopictus* in the laboratory' has been submitted for publication.

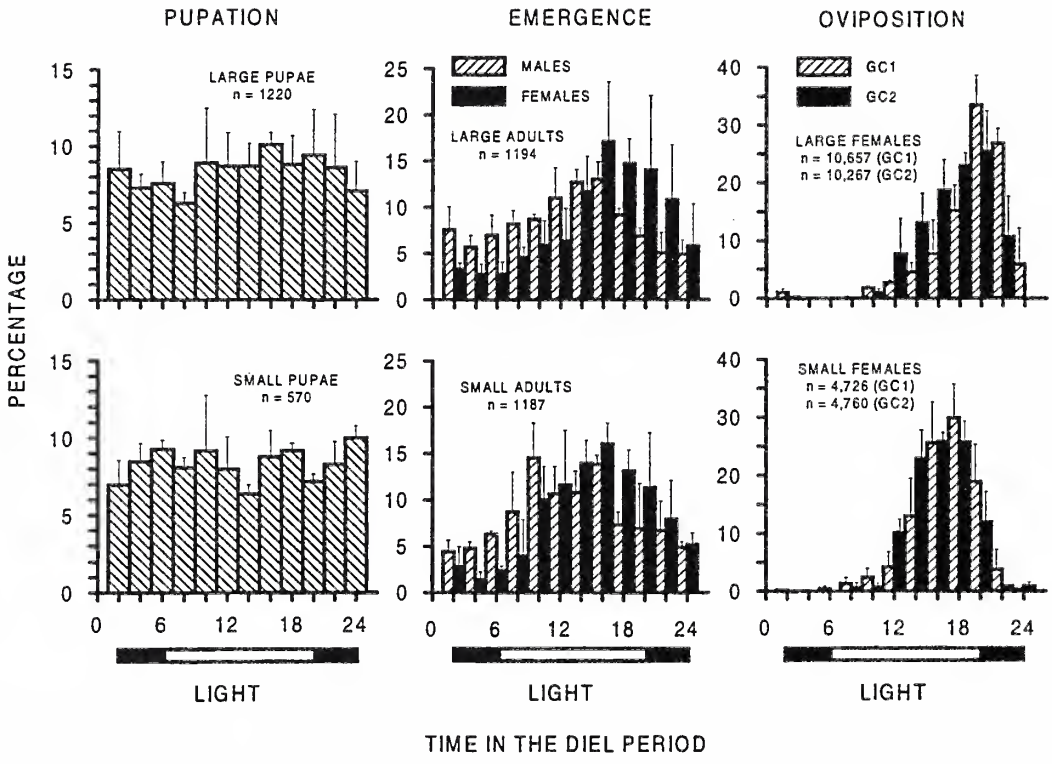


Figure 1

Mean percentage pupation, male and female emergence, and oviposition during 2 gonotrophic cycles (GC1, GC2) in large and small *Aedes albopictus*, by time of observation in the diel period. Vertical bar is one standard error of the n.

CRIS-6615-32000-032-00D

MOSQUITO AND NON-TARGET HOST RANGE OF A MERMITHID NEMATODE

James J. Becnel

Objective: To determine the mosquito and non-target host range of the mermithid nematode *Strelkovimermis spiculatus*.

Methods: Mosquito Host Range: The following mosquitoes were obtained from the colony at the USDA, ARS Gainesville, FL; *Aedes aegypti, Ae. albopictus, Ae. taeniorynchus*, *Anopheles quadrimaculatus. Toxorhynchites rutilus septentrionalis, Culex quinquefsciatus.* One family of *Cx. restuans* was collected from the field. All mosquitoes were exposed to preparasites in groups of 100 except for *T. r. septentrionalis* which were exposed in one group of 25. These larvae were exposed to *Strelkovimermis spiculatus* preparasites at a dose of 5 preparasites to one larva in 100 ml of deionized water. An equal number of mosquitoes were not exposed to preparasites and served as controls. After 24 hours, larvae were transferred to 500ml pans and reared to 4th instar. Ae. *taeniorynchus* were exposed and reared in 1% NaCl solution and preparasites were hatched in 1% NaCl solution. After mosquitoes reached the 4th instar, larvae were individually placed in well plates. Pupation, death, and number of nematodes were recorded. Nontarget Tests: *Corethrella bracklei* larvae (mixed ages second to fourth instar) were collected from a flooded ditch in Micanopy, Florida. *Chironimus* sp. were collected from artificial cement containers at the USDA, ARS Gainesville, Florida. All other nontarget insects were collected from secondary treatment ponds at a swine facility in Gainesville, Florida. All nontargets were exposed to 5 preparasites per individual. An equal number of nontargets not exposed to nematodes served as controls. After one week, mortality and infection was recorded.

Results: All of the mosquitoes exposed to *S. spiculatus* were susceptible (Table 1). *Culex quinqufisciatus* was the best host for this parasite while *An. quadrimaculatus* and *T. r. septentrionalis* were poor hosts. None of the nontarget hosts were susceptible to infection by *S. spiculatus*.

Plans: This data documenting the specificity of this parasite for mosquitoes will be published as part of the project ot evaluate *S. spiculatus* in the field.

Table 1. Mosquito Host Range of *Strelkovimermis spiculatus*

SPECIES	n	% mortality	% infected	Average Nematodes per Larva
An. quadramaculatus	*300*	100	16	1.1
Aedes aegypti	*300*	74	68	1.3
Ae. albopictus	*300*	52	52	1.6
Ae. taeniorynchus *	*300*	92	80	1.1
Ae. triseriatus	*300*	84	42	1.5
Culex quinquafasciatus	*300*	95	88	2.1
Cx. restuans	100	83	35	1
Toxorhynchites rutilus septentrionalis	*25*	58	20	1

**Exposed in 1 ppt NaCl*

Table 2. Nontarget Tests with *Strelkovimermis spiculatus*

SPECIES	n	% mortality	% infected	Average Nematodes per Larva
Corethrella bracklei	50	5	0	0
Copepods (mixed species)	200	0	0	0
Chironomus sp.	50	0	0	0
Rat-tailed maggot	8	0	0	0
Small aquatic beetles	50	0	0	0
Medium aquatic beetles	30	0	0	0
Damselfly larvae	15	0	0	0
Dragonfly larvae	10	0	0	0

SURVIVAL OF EDHAZARDIA AEDIS SPORES IN THE FIELD

James J. Becnel

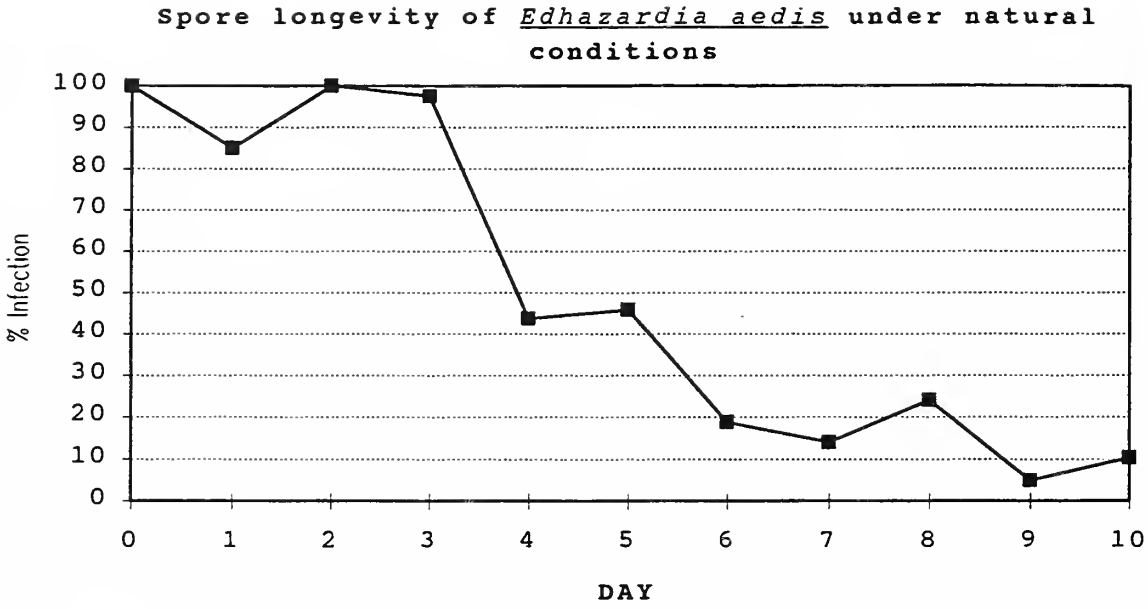
Objective: To determine the survival of *Edhazardia aedis* spores in the field. Survival of the horizontally infectious spores is important in designing strategies to introduce this parasite into populations of *Aedes aegypti*.

Methods: Plastic buckets with 15 liters of well water plus 200 grams of autoclaved leaves were allowed to steep for 3 days. The infusion was stained through a 230 mesh sieve and one liter quantities placed into each of 33 plastic containers. Twenty-two of the 33 containers were each contaminated with 3 *Edhazardia aedis* infected *Aedes aegypti* larvae (1.4 X 10^6 spores/container). Three healthy *Ae. aegypti* larvae were added to each of the remaining 11 containers. Each day, 2 exposed and 1 control container were selected into which 20 larvae (48 hour old) were placed. Larvae were removed after 4 days, counted and individually smeared and stained with Giemsa to determine percent infection. Two tests were conducted, one in October, 1995 and one in May, 1996.

Results: Spore survival remained high for the first 3 days of the study with infection levels approaching 100% (Fig. 1). On days 4 and 5, infection levels decreased to approximately 50%. After 10 days infection levels decreased to 10%.

Plans: This phase of the study has been completed and a manuscript is in preparation.

FIGURE 1.


Spore longevity of Edhazardia aedis under natural conditions
% Infection
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
DAY

FIELD EVALUATION OF *EDHAZARDIA AEDIS* AGAINST *AEDES AEGYPTI* IN FLORIDA

James J. Becnel

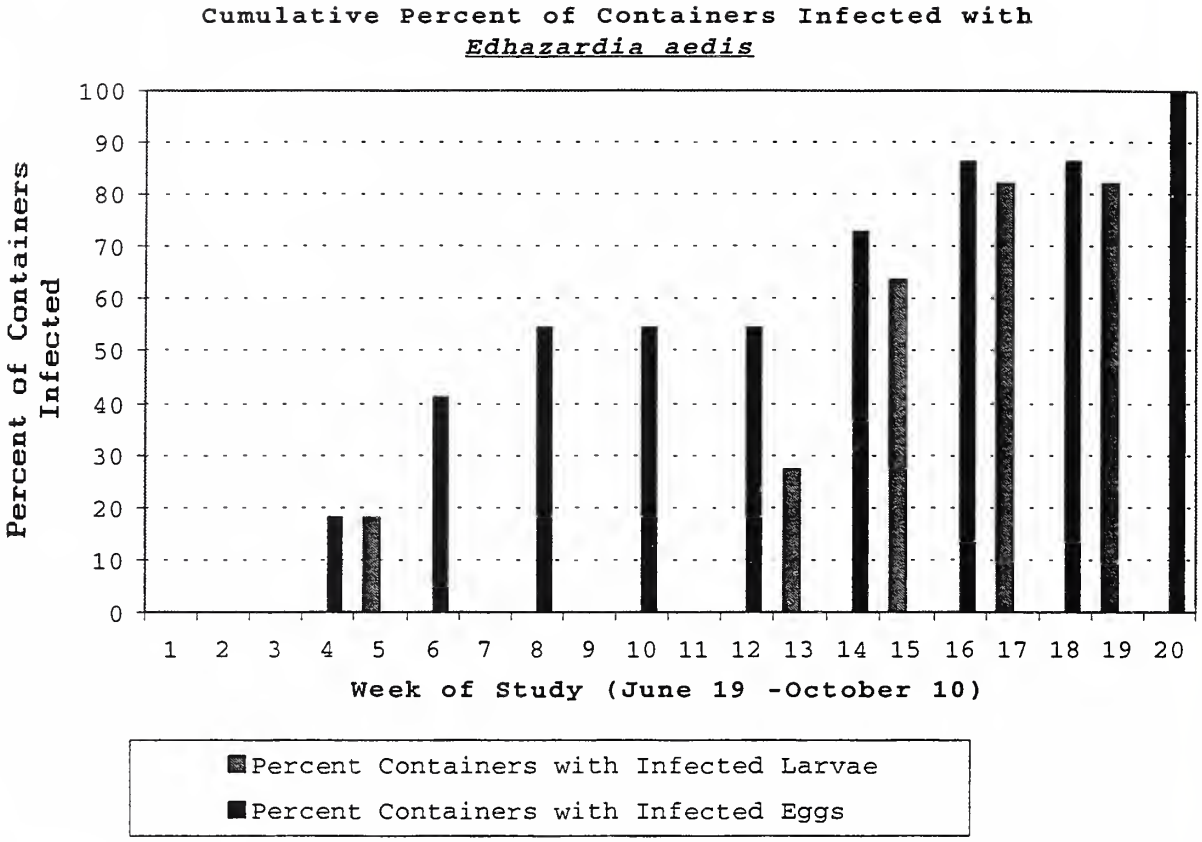
Objective: To measure the persistence and dispersal of the parasite *Edhazardia aedis* within a field population of *Aedes aegypti* in Florida.

Methods: Tests were conducted in a 10 X 30 meter screened enclosure located in Gainesville, FL. Four rows of golf cart tires (26 total) were positioned on racks 1 meter off the ground. One gallon plastic containers were placed into each tire to which autoclaved leaves and 1 liter of well water were added. Paper strips (19 X 12.5 cm) lined each container to provide for oviposition. Two rabbits in 1 X 3 meter cages were located in corners of the enclosure opposite one another and served as a blood source. Approximately 5500 pupae of "wild" *Ae. aegypti* (8 generations in the laboratory) were allowed to emerge into the cage. After 4 weeks, larval populations were present in all containers. Two containers were randomly selected from each row (8 total). One container from each row was selected to serve as covered controls; two of these were contaminated with 6 decapitated *Ae. aegypti* larvae infected with *E. aedis* and two with 6 decapitated healthy *Ae. aegypti*. The other selected container from each row was contaminated with 6 decapitated infected *Ae. aegypti* larvae and left uncovered. Pupae were removed from the covered containers daily, set up individually in vials for emergence and held for 48 hours. The sex was recorded and the infection status of each adult was determined by the presence of spores. All dead individuals were examined for infection. Sampling of open containers began 4 weeks post inoculation. Egg papers were collected biweekly and cut in half; one half was returned to the container and flooded, the other half hatched in the laboratory, reared to the fourth instar and examined for vertical infection. The number of larvae in each container was determined at week 5 and then biweekly, starting on week 13. The number of patent infections (either vertically or horizontally induced) was recorded in each observation period.

Results: A total of 558 adults emerged from the 2 unexposed closed containers with a 0.7 % mortality rate. In the 2 exposed closed containers, a total of 335 adults emerged with a mortality rate of 29 %. This difference represents a decrease in emergence of 40 % caused by the presence of the parasite. More importantly, however, 74.8% of all female adults that emerged from the exposed containers were infected with *E. aedis*. These infected females are capable of vertically transmitting the parasite to progeny. Indeed, spread from the originally infected containers to the other containers in the test resulted in 100% of the containers being positive for infected eggs 20 weeks after the original introduction (Fig. 1). Examination of the individuals from each container paralleled the ovipositional data with over 80% of the containers infected after 20 weeks (Fig. 1). The original 4 contaminated containers were positive throughout the sampling time indicating an ability of the parasite to recycle and persist.

Plans: This study is ongoing and will be continued until mosquito activity is ended by cold weather. Follow-up studies in the spring will determine the the overwintering success of the parasite.

FIGURE 1.


Cumulative Percent of Containers Infected with
Edhazardia aedis
Percent of Containers Infected
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Week of Study (June 19 -October 10)
Percent Containers with Infected Larvae
Percent Containers with Infected Eggs

SEX AND AGGREGATION PHEROMONES IN FILTH FLIES RELATED TO Musca domestica.

D.A. Carlson and U.R. Bernier

Objective: Control or reduction of filth-breeding flies is a desirable goal during deployment of US Armed Forces to the Middle East and around the Pacific Basin, but the efficacy of baits against species of filth flies encountered in the Middle East and Asia is unknown. The long-term objective is to increase the efficacy of fly-lure baits with attractive non-toxic chemicals.

Methods: In a cooperative effort with LCDRs S. Cope and G. Tetreault, collections of filth-breeding Muscidae including fresh *M. d. domestica s.str., M. domestica calleva, M. d. curviforceps*, *M. sorbens, M. biseta* and *M. vetustissima* flies were collected by U.S. Military entomologists in Egypt, Zanzibar, Kenya, Viet Nam, Guam, and Hawaii. A shipping methodology that included using 99-well plastic immunoplates was successful and did not require pinning of insects in the field. Brief drying allowed successful shipment of individual flies without loss of hairs or heads. We received specimens for identification of *M. d. domestica* and the closely related sub-species*M. d. calleva, M. d. curviforceps,* also *M. sorbens* and the closely related *M. biseta* and *M. vetustissima,* the Australian Bush fly. Extraction of hydrocarbons from individual specimens were done in Gainesville for GC and GC-MS.

Results: About 250 new hydrocarbon profiles were obtained by GC, but 100 new samples have not been processed. GC-MS has been completed on some. All flies possess long chain alkanes, methyl- and lesser amounts of dimethyl-branched alkanes. There are also small amounts of alkenes at C23, C27, C29 and C31. It appears that some housefly strains from Egypt, Okinawa and Viet Nam are similar to USDA colony flies. *Musca d. domestica* s.str. have been recently identified from many locations in Egypt. *Musca d. curviforceps* females are different from *M.d. domestica*. However, novel alkenes were found in *M. biseta* in the C33 and C35 region that were similar to the *M. sorbens* and *M. vetustissima* females.

Plans: We hope to show that other fly species may be attracted to *M. domestica* pheromone fly lures, as some baits have food- or filth-type components. Improvements to baits by addition of novel synthetic alkenes as pheromone candidates is possible but the composition of sex or aggregation pheromones in other fly species is unknown. These alkenes can be readily determined with available knowledge and equipment using dead, dried flies mailed to USDA-CMAVE for chemical analysis.

DISTINCTIVE HYDROCARBONS OF THE PARASITOID *MUSCIDIFURAX* (HYMENOPTERA: PTEROMALIDAE)

D.A. Carlson, C.J. Geden, and U.R. Bernier

Objective: Parasitoid species that have been introduced or are contemplated for release within the USA are often difficult to identify by conventional morphology. Since genetic markers for confirming the identities of specimens in culture are currently unavailable, cuticular hydrocarbon patterns (CHP) of *Muscidifurax raptor*, *M. zaraptor*, *M. raptorellus*, and *M. uniraptor* were examined to compare and determine the utility of CHP for easy identification of species and gender.

Methods: Insects were collected, frozen, and extracted with hexane solvent. The hydrocarbons obtained by chromatography were analyzed by gas-liquid chromatography. Data were collected and the mean per cent composition was determined for all GC peaks. These data may be examined and analyzed using simple peak ratios or one of the four major types of pattern recognition methodologies: mapping and display, clustering, discriminant analysis, or principal components modeling.

Results: GC of the hydrocarbons from pooled and individual insects of closely related species gave reproducible patterns that clearly differentiated between the related species *Muscidifurax raptor*, *M. zaraptor*, *M. raptorellus*, and *M. uniraptor.* Pronounced sexual dimorphism in the patterns was also present. Patterns were consistent within species using colony material collected world-wide. Mass spectra of novel and rare methyl-branched alkanes were described for confirmation.

Plans: We will complete the study, and compare gregarious versus solitary *M. raptorellus* from South America. At present, there are about a dozen common species of pteromalid parasitoids of muscoid flies in the US. Under certain conditions, parasitoid releases can be helpful in regulating populations of flies in animal production systems. Although most of the indigenous parasitoids of flies can be identified readily by morphological characters, there is a need for clearer taxonomic characters to aid identification of exotic species that are candidates for release in the continential US. For example, recent introductions of the tropical parasitoids, *M. raptorellus* and *M. uniraptor,* into California underscore the need for better taxonomic characters for identifying species in this morphologically parsimonious genus.

PEPTIDE HORMONE MIMICS OF TRYPSIN-MODULATING OOSTATIC FACTOR

D.A. Carlson and L. Okedi

Objective: To develop new species-specific methods of insect control that are based on the use of natural, biorational processes and which interfere with insect development, particularly for blood-feeding insects. To design and develop peptide mimics that effectively sterilize blood-feeding insects using non-hazardous materials.

Methods: Synthetic peptide mimic compounds were injected in microgram quantities into living blood-fed female mosquitoes in an attempt to interfere with egg yolk synthesis and to prevent yolk transfer to the undeveloped eggs. Also, adult female stableflies were treated with Trypsin-Modulating Oostatic Hormone (TMOF) related compounds dissolved in DMSO/acetone. Egg development was determined by direct measurement; toxic effects with some compounds was noted.

Results: Six novel non-peptide mimics of peptide hormones based on patented TMOF peptide hormones, were synthesized then showed to have potent sterilizing activity on mosquitoes and stable flies. Trypsin-like enzyme synthesis was significantly inhibited with 5 microgram treatments of four of the most effective pepide mimics. Because the blood meal in treated insects is undigested or poorly digested, egg yolk protein is not synthesized by the fat body or transferred to the ovaries, and the eggs do not mature. Curiously, and for unknown reasons, some insects in a test group were not affected. In stableflies treated with selected peptides, eggs do not accumulate egg yolk, and do not grow, therefore, the females are sterile.

Plans: We will complete the dose-response studies with these compounds. The results will be used to design other peptide mimics that are lipophilic and that should be more easily carried across the insect cuticle in effective quantities. This research could lead to novel control methods for blood-feeding insects, particularly near livestock facilities, where area treatment, attractive traps, or the use of autocidal methods also could be deployed.

Anopheles quadrimaculatus SPECIES COMPLEX IDENTIFICATION

D.A. Carlson, J.R. Reinert and J.A. Seawright

Objective: To utilize extracted surface hydrocarbons of newly-emerged individual Type isofemale brood specimens of the *Anopheles quadrimaculatus* species complex A, B, C1, C2 and D to develop a rapid and effective non-destructive identification method is needed.

Methods: Insects were reared and extracted for analysis and the hydrocarbons analyzed by gas-liquid chromatography. The data was collected and mean per cent composition was determined for 40 peaks (characters KI 2300 -KI3300) for each subspecies, and additionally for three specimens of each subspecies used as a type.

Results: The means for each character appeared to overlap, but a set of characters that would give useful classification of the individual mosquitoes was determined. An additional set of data has been investigated for representative specimens to determine if higher molecular-weight materials will show any differences, as was the case for the *Anopheles gambiae/An. arabiensis* species complex that used 2 peak ratios, R1=KI 3950/3300 and R2=KI 3950/4150. Data collected from Type isofemale brood adults appear to separate A and B from C1 and from the others and D from the others, respectively.

Plans: It may be possible to find identifying characters in older, sexually mature females.

An. quadrimaculatus SPECIES C1 HATCHING FACTOR

D.A. Carlson, G. White and U.R. Bernier

Objective: To isolate and identify the *An. quadrimaculatus* Species C1 Hatching Factor. Discovery of the chemical(s) involved could explain the egg- hatch mechanism in some floodwater mosquitoes. Knowledge of a chemical structure and mode of action could lead to novel methods of mosquito control that are environmentally safe. Inhibition of egg hatch could be a significant breakthrough if applicable to other species.

Methods: A Hatching Factor was chemically extracted and isolated from swamp water. Aliquots of extracts and fractions were spotted on filter paper and dried, then added to mosquito eggs in beakers in 25 ml of distilled water. These eggs did not hatch well in fresh, tap or distilled water. However, swamp water from the Suwannee River basin was extracted with hexane solvent and the crude extract fractionated on a column to give an active 20% ether fraction. Active fractions caused hatching within a few minutes when added the water and eggs in the beaker. Recently, an active extract that was recovered from a hexane extraction of swamp soil, was fractionated by high performance liquid chromatography using 70% methanol in water; the activity was recovered in early fractions.

Results: The odorous yellow oil obtained by extraction contains many compounds, as shown by TLC after column chromatography, including compounds that are produced by fungi, and which smell of moist earth and swamp muck. Although HPLC-MS and GC-MS have so far failed to give good indicators of candidate compounds other than small amounts of phthalate contaminants, the active compound appears chemically stable and potent, but is present in small amounts. It is not yet known if the odorous compounds are responsible for biological activity. Several compounds identified from garden soil appear in the literature, including 3-isopropyl-2-methyoxypyrazine, methyl isoborneol and geosmine. The active material exhibiting biological activity appears to be semi-polar and relatively non-volatile.

Plans: Repeat the isolation and separation steps when mosquitoes are available next spring and complete the chemical identification. Investigate the mechanism of action of hatching factor.

EVIDENCE FOR A NEW SIBLING SPECIES OF THE ANOPHELES QUADRIMACULATUS COMPLEX

A.F. Cockburn

Objective: To use genetic techniques to increase the efficiency of mosquito control operations by differentiating pest and non-pest mosquito species.

Methods: Meta-analysis of previous studies of the Anopheles quadrimaculatus species C1 and C2; including isozyme electrophoresis, polytene chromosome cytology, genetic crosses, and DNA restriction analysis.

Results: Several different types of experiments, including isozyme electrophoresis (published by Dr. S. Narang), genetic crosses (conducted by P. Kaiser), and mtDNA restriction analysis (conducted by Dr. S. Mitchell) all showed an excess of variability in Anopheles quadrimaculatus species C2, suggesting that this species might be a species complex. Dr. Mitchell also conducted rDNA restriction analysis of this taxon, however she only included individuals having one type of mtDNA. Since these studies were conducted at different times on different samples, it is not possible to determine from the existing data whether the trend in these studies is due to the presence of more than one species. This result demonstrates the importance of a multidisciplinary approach to sibling species identification.

Plans: No further work is planned on this project.

MOLECULAR BIOLOGICAL CHARACTERIZATION OF POTENTIAL VIRAL BIOLOGICAL CONTROL AGENTS FOR MOSQUITOES

A.F. Cockburn, J. Becnel, B. Moser, J. Maruniak[1]

Objective: To develop a molecular biological techniques to analyze mosquito viruses.

Methods: DNA was isolated from mosquito iridescence virus (MIV) and mosquito baculovirus (MBV). A recombinant DNA library was constructed from MIV. A selection of restriction enzymes were tested to determine how many times they cleaved the MIV genome.

Results: Clones were isolated from the MIV library that contained single restriction fragments. These were plaque purified and screened by DNA hybridization to verify that they contained MIV DNA. Individual clones were stored in glycerol culture for future use. Restriction enzymes that cut the MIV genome five to ten times were chosen for further analysis. Southern blots containing restricted MIV DNA were prepared.

Plans: The individual clones will be used to probe the MIV southern blots. This information will be used to construct a restriction map of the MIV genome. The same techniques will be used to construct a restriction map of the MBV genome.

[1]Department of Entomology and Nematology, University of Florida

GERMINATION OF *NOSEMA ALGERAE* (MICROSPORA) SPORES: CONDITIONAL INHIBITION BY D_2O, ETHANOL AND HG^{2+} SUGGESTS DEPENDENCE OF WATER INFLUX UPON MEMBRANE HYDRATION AND SPECIFIC TRANSMEMBRANE PATHWAYS

E. Frixione[1], L. Ruiz[1], J. Cerbón[1] and A.H. Undeen

Objective: To determine the role of water-flow across the spore wall and plasmlemma in the germination of microsporidian (Protozoa) spores.

Methods: Density gradient purified spores of *N. algerae* from *Helicoverpa zea* larvae were stimulated to germinate in 100 mM NaCl; pH 9.5, 20 mM tris-borate. Inhibition of germination by additions or substitutions to this medium with D_2O and low concentrations of ethanol or Hg^{+2}, agents known to affect water-flow across membranes, were tested. Germination under the various experimental conditions was assayed by mixing, directly on microscope slides, 5 µl of spore suspension (2×10^7 spores/ml) in distilled water or D_20 with 5 µl of the test solution at twice the desired final concentrations and observed at 400X with phase-contrast microscope. The temperature at the specimen area on the microscope stage was monitored with an attached thermistor probe and regulated by the positioning of two incandescent lamps. Experiments requiring temperatures below ambient were carried out similarly in a cold room. Numbers of discharged and undischarged spores within the field of view were rapidly counted using a laboratory double-key counter. Mean % germination was obtained from at least four replicates under each condition tested.

Results: Replacement of common water with D_20 inhibited spore germination as observed by a delayed response to the stimulus and decreased final percentages of germinated spores, an effect enhanced by previous storage of the spores for 1 week at 4° C in 100% D_20. Increased temperature or ionic concentration ameliorated inhibition by D_20. Inhibition by D_20 was readily reversible by returning spores to water. Germination dropped from about 80% to around 50% in the presence of 2% (434 mM) and nearly abolished by 4% ethanol in a stimulation solution with the normal level of salt. Doubling the concentration of NaCl in the 2% ethanol-medium only marginally increased the response at 30° C. Germination was little improved by a 4-fold increase of NaCl. Damage to the spores by the alcohol can be ruled out, because exposure for 20 min to 4% ethanol in water or in the normal stimulation solution did not affect germination upon tranfer to ethanol-free stimulation medium. Germination was inhibited by low levels (100-250 µM) of $HgCl_2$. The blockade by Hg^{2+} was not modified by increased temperature. High percentages of spores germinated in 250 µM $HgCl_2$ with NaCl elevated to 400-800 mM but germination was considerably delayed. The effect of Hg^{2+} was reversible; over 50% of spores pre-treated for 1 min with 250 µM $HgCl_2$ germinated within 5 min in the normal stimulation solution following a couple of brief rinses with distilled water. 2-mercaptoethanol, when added to the rinse water, improved recovery and, when added to the $HgCl_2$-containing stimulation solution, prevented the inhibition.

Plans: Continuation of this work will focus on the evaluation of the effects of these and other inhibitors on germination rates of individual spores using videomicroscopic techniques.

[1] Centro de Investigación y de Estudios Avanzados del IPN, Apartado Postal 14-740, México D.F. 07000

LABORATORY STUDIES OF *STRELKOVIMERMIS SPICULATUS,* A NEMATODE PARASITE OF MOSQUITOES

T. Fukuda, S.E. White, O.R. Willis and D.R. Barnard

Objective: To determine the effects of various conditions in the laboratory on the rearing of *Strelkovimermis spiculatus,* a nematode parasite of mosquitoes.

Methods: The mermithid nematode, *Strelkovimermis spiculatus* was exposed to the mosquito hosts, *Aedes aegypti, Anopheles quadrimaculatus,* and *Culex quinquefasciatus* under the following sets of conditions: (1) different ratios of preparasites to larvae (1, 2, 4, 8, 16:1), (2)different exposure times (0.5, 1, 2, 4, 8, and 24h), (3) variable preparasite age (0-10 days, held at 5 and 24°C , and (4) different host ages (1-5 days). Five days after exposure the mosquito larvae were examined and the percentage infection of hosts recorded.

Results: Overall *Cx. quinquefasciatus* proved to be the best host for rearing *S. spiculatus,* with a mean percent infection of 70%. *Anopheles quadrimaculatus* was not a suitable host. To produce 50% infections in *Cx. quinquefasciatus* a ratio of 1.7:1 (preparasite:larvae) was required using larvae 1.1 days or younger. Although the overall infection levels of mosquitoes did not change, the ability of preparasites to infect mosquitoes was extended to 7 days when held at 5°C compared with 2 days at 24°C. The extension of exposure times from 0.5 to 24h did not significantly effect the percentage of infection in any of the hosts, which showed that the majority of infections in mosquitoes occurred during the first half hour of exposure to the preparsites. *Culex quinquefasciatus* produced the most adult nematodes under all conditions. As a result, this species is the host for the mass production of *S. spiculatus* at this laboratory.

Plans: This phase of the study has been completed, and the data will be presented in a manuscript prepared for the Journal of the American Mosquito Association.

EFFECTS OF EXPOSURE CONDITIONS ON *STRELKOVIMERMIS SPICULATUS* ON THREE MOSQUITO HOSTS.

Test Parameter	*Ae. aegypti*	*An. quadrimaculatus*	*Cx. quinquefasciatus*
Ratio			
Mean Percent Infection	48.6 ± 8.4[B]	15.3 ± 4.3[C]	69.7 ± 6.5[A]
$Ratio_{50}$	4.4 (4.0, 4.9)	14.1 (9.2, 31.3)	1.7 (1.4, 2.0)
Adults Expected at $Ratio_{50}$	27.3	30.6	43.4
Exposure Time	Not significant for Percentage Infection		
Mean Percent Infection	58.9 ± 4.7[B]	7 ± 1.4[C]	83.5 ± 3.1[A]
Larval Age			
Mean Percent Infection	21.4 ± 4.8[B]	2.2 ± 1.0[C]	49.8 ± 6.7[A]
Larval Age_{50}	0.08 (0, 1.1)	-	27.1 (19.8, 34.4)
Adults Expected at Larval Age_{50}	26	-	92
Preparasite Age and Storage Temperature			
5 °C			
Mean Percent Infection	34.5 ± 6.1[B]	5.7 ± 1.9[C]	48.5 ± 6.1[A]
Preparasite Age_{50}	14.3 (6.6, 22.6)	-	170.7 (130.0, 254)
Adults Expected at Preparasite Age_{50}	22	-	44
24 °C			
Mean Percent Infection	26.9 ± 6.3[B]	3.7 ± 1.7[C]	48.5 ± 8.6[A]
Age_{50}	18.8 (15.2, 22.6)	-	73.2 (61.0, 86.0)
Adults Expected at Preparasite Age_{50}	39	-	51

PARAIOTONCHIUM MUSCADOMESTICAE (NEMATODA: TYLENCHIDA: IOTONCHIDAE) INFECTING HOUSE FLY: DOSE-RESPONSE RELATIONSHIP, EFFECT ON LONGEVITY, AND PERSISTENCE OF GAMOGENETIC NEMATODES OUTSIDE THE HOST

C.J. Geden

Objectives: To develop optimal nematode:host ratios for consistent parasite production, to determine residual efficacy of nematodes outside the host, and to evaluate the effect of infection on fly longevity.

Methods: Dose response bioassays were conducted by first adding 250 cm^3 of fresh rearing medium to screen-topped 350-ml plastic cups. Fly eggs (100/cup) were added to the cups, then nematodes were dissected from infected flies and inoculated into the medium at doses ranging from 0.001 to 10.0 "infected fly equivalents" (IFE) per cup, plus uninfected controls. On average, 1 IFE=30,000 nematodes. Cups were held for 7 days then the fly pupae were counted, weighed, and placed in small fly cages provided with food and water. Flies were counted and dissected to determine their infection status 10-14 days after emergence. Nematode counts were made in infected flies. Subsamples of infected flies (and uninfected controls) were held and monitored for longevity.

For trials of nematode latency and residual efficacy, nematodes were first inoculated into cups of rearing medium at doses of 0, 0.1 and 1.0 IFE. Fly larvae were then added to the cups (50 larvae/cup, 3 cups per dose and time interval) at the following times relative to inoculation with nematodes: 24 h before inoculation, the same day, and on days 1, 2, 3, 5, 7, and 10 after inoculation. As before, pupae from each cup were counted, weighed, and held for fly emergence and determination of infection status 10-14 days after emergence.

Results: Infection levels ranged from 1.7 at 30 nematodes per cup to 100% at 300,000 nematodes (10 IFE's). High larval and pupal mortality was observed among flies at the high dose. Infected adult flies of both sexes lived about half as long as uninfected flies. Examination of nematode age structure in infected flies revealed that mature gamogenetic females produce about 8 parthenogenetic females each, regardless of nematode crowding levels. In contrast, production of new gamogenetic nematodes by parthenogenic females was density-dependent, with average reproductive rates ranging from 1,627 progeny per parthenogenetic female, at 8 females per host, to 330 at 83 females per host. Infected flies contained 12,000-45,000 new gamogenetic nematodes 10-14 days after fly emergence. Male flies were as susceptible to infection as females. Gamogenetic nematodes required 24 h after deposition into fly larval rearing medium before they were capable of infecting new host larvae. Nematodes persisted in rearing medium for 3-5 d after deposition; no infections were observed at 7 and 10 d after deposition.

Plans: This phase of the study has been completed and a manuscript with the above title has been sumitted to "Biological Control" for publication.

DEVELOPMENT MODELS FOR THE FILTH FLY PARASITOIDS *SPALANGIA GEMINA*, *S. CAMERONI* AND *MUSCIDIFURAX RAPTOR* UNDER CONSTANT AND VARIABLE TEMPERATURES

C.J. Geden

Objectives: To evaluate temperature constraints on development of the Brazilian parasitoid *Spalangia gemina* compared with two indigenous species.

Methods: House fly pupae were first exposed for 24h to 5-day-old female parasitoids, then the parasitized pupae were transferred to environmental chambers maintained at 15, 20, 25, 27.5, 30, 32.5, and 35°C. In addition, tests under variable temperature conditions were conducted to simulate outdoor summer conditions in Florida by placing parasitized pupae in an environmental chamber programmed to fluctuate daily from 24 to 36°C. Humidity was kept constant at 80% and a 14L:10D photoperiod was used.

Development rates at constant temperatures were modeled using two algorithms, the biophysical model of Sharpe and DeMichele and the computationally simpler degree-day method. Parameter estimates for the Sharpe and DeMichele model were obtained by non-linear regression. For the day-degree model, minimum developmental threshold temperatures were calculated by the X-intercept method, and the number of degree-days for each species determined as the mean of the degree-days required at each constant temperature over the linear portion of the temperature response (15 to 30°C). The two models were then compared for their effectiveness at predicting development under fluctuating conditions.

Results: *Muscidifurax raptor* Girault and Sanders was the fastest developing species, with females completing development in 13.8 days at 32.5°C, to 66.5 days at 15 C. *Spalangia gemina* Boucek females completed development in 20.8 days at 30.0°C to 161 days at 15.0°C, whereas *S. cameroni* Perkins females completed development in 20.6 days at 30.0°C to 155.5 days at 15.0 C. Male development times were 90.3% those of females for *S. gemina*, and 92.7 and 88.6% those of females for *S. cameroni* and *M. raptor*, respectively. Parasitoid survival was very low at 35°C for all species, and no *Spalangia* survived constant exposure to 15.0°C. Exposure to these lethal temperatures for shorter periods indicated that the parasitoids can tolerate them well under conditions more typical of the field. Neither type of model was superior for all three species because of interspecific differences in the parasitoids' responses to high temperatures. Agreement between predicted and observed development times for all three species was optimized by making small empirical adjustments of a key parameter in the biophysical model.

Plans: This study has been completed.

DEVELOPMENT OF HYDROTAEA AENESCENS IN THE MANURE OF UNWEANED DAIRY CALVES

J.A. Hogsette and R. Farkas

Objective: To determine the ability of *Hydrotaea aenescens* larvae to develop in manure of unweaned calves.

Methods: Manure was collected from 1-8 week-old dairy calves, frozen (between -4°C and -20°C) then warmed to room temperature before use. Moisture content of the manure was determined by drying it in an oven at 55°C for 48 hours. To assess fly development, manure samples (100 g) were placed in 240-ml plastic cups and 50 1st-instar *H. aenescens* larvae added to each cup. The cups were covered with cloth, to prevent the escape of larvae, and maintained at 26°± 2°C. After 7 - 10 days, the cups were inspected for pupae, which were separated from the manure, counted, weighed, and then held until adult eclosion. Parallel tests were performed in Gainesville and in Budapest, Hungary.

Results: In Gainesville, the more *H. aenescens* developed in manure from 2-week-old calves, with a slight decline in the numbers of flies produced as calf age increased to 8 weeks. Fly development was poorest in manure from 1-week-old calves. In Hungary, the most flies developed in manure from 4-week-old calves, but the numbers declined as calf age reached 9 weeks. The poorest fly production was in manure from calves 1-3 weeks old. Pupal weights averaged ca. 16.5 mg, and were similar in tests from Gainesville and Hungary. Our results demonstrate that *H. aenescens* larvae can utilize manure from unweaned calves.

Plans: These studies are near completion. Additional studies are planned using manure from adult cattle.

EFFECTS OF CONSTANT EXPOSURE TO ULTRAVIOLET LIGHT FROM INSECT LIGHT TRAPS ON THE GROWTH OF BROILER CHICKS

J.A. Hogsette and H.R. Wilson

Objective: To determine whether ultraviolet (UV) light from electric fly traps alters the rate of development of broiler chicks when traps are illuminated for periods beyond the designated lighting schedule.

Methods: One hundred 2-day-old broiler chicks were housed in each of four experimental houses at the University of Florida Poultry Science Farm. UV lights from electrocutor-grid insect traps were illuminated in 2 houses (treated) for the duration of the test. The remaining two houses (control) plus the two treated houses received supplemental fluorescent lighting set at the industry lighting standard. Total mortality, feed consumption, and body weight of chickens was measured at 0, 3, and 6 weeks. The test was terminated after 6-weeks. Subsequently, light traps were moved so that the control houses became the treated houses and vice versa. One hundred additional 2-day-old broiler chicks were housed in each of four houses, and the test was repeated.

Results: Differences in mortality between treated (1.5%) and untreated (3.9%) groups of broilers were small and within the expected range. Final bird weights after 6 weeks were 2.028 kg (treated) and 2.070 kg (control). The most interesting difference was feed consumption. Treated birds consumed 0.09 kg less feed than untreated birds, which represents a savings of 90 kg per 1,000 birds. These results indicate that constant exposure to UV light from insect traps does not adversely affect growing broiler chicks, and may actually increase feed efficiency.

Plans: This study has been completed. An additional study in commercial broiler houses may be conducted in the future.

RESTRICTED INGESTION OF BACTERIA BY FIRE ANTS

D.P. Jouvenaz, J.C. Lord, and A.H. Undeen

Objective: The imported fire ants, *Solenopsis invicta* and *Solenopsis richteri*, workers and queens feed only on liquids. Pathogenic bacteria might be used against fire ants but, unless cells or spores are actually ingested by the queens, they are not likely to become infected. This study tests whether fire ant queens ingest bacteria.

Methods: Three species of bacteria were chosen for this study, *Serratia marcescens*, *Bacillus thuringiensis* var. *israelensis*, and *Bacillus sphaericus*. Each of these bacteria were mixed with boiled egg yolk to a paste consistency and fed daily for 14 days to small polygene colonies of fire ants. Daily, for the last ten days of the test, each colony was also fed one living corn earworm larva immediately after the larva had been injected with 0.25 ml of bacterial cell or spore suspension. The ants readily consumed the diet as we observed daily and confirmed by isolating *S. marcescens* from the midgut of fourth instar larvae. Each ant colony consisted of 12 queens, approximately 5,000 workers and 1.5 g of brood. Colonies were maintained in soil-free nests at 28°C in a walk-in rearing chamber. Ten queens from each colony were surface-sterilized and dissected aseptically, first removing the head and venom gland. A similar procedure was performed on workers. Homogenates of gastric guts of ants were cultured on appropriate nutrient agar at 28° C and examined 24 and 48 hr later. *Serratia marcescens* was identified by red-pigmented colonies, *B. sphaericus* by the swollen, terminal spore and toxicity to mosquito larvae and, bacteria having parasporal bodies were considered to be *B. thuringiensis*.

Upon further incubation, minute colonies of bacteria developed on several plates which had initially appeared to be sterile. 58 queens were collected from field colonies and examined within 4 h. One colony from each queen was subcultured and maintained for characterization. Gram-stained cells (N = 50) were measured with a Vickers-A.E.I. splitting image micrometer. Isolates of the bacterium were analyzed with a computerized bacterial identification system (BioLog Inc., Hayward, CA).

Results: Queens, workers and brood mortality was comparable to that of control insect colonies. *Serratia marcescens*, the smallest bacterium, was not recovered from any of the 20 queens and 10 workers. *Bacillus sphaericus* was recovered from the gaster of one of 20 queens and none of the workers. *Bacillus thuringiensis* was not recovered from any of the 20 queens but was found in 3 of the 10 workers.

A slow-growing bacterium was isolated from (15%) of the 60 queens from test colonies and from (13.8%) of the 58 queens from field colonies. This bacterium is a Gram negative, non-motile, facultative anaerobe measuring about 0.5 x 0.5 - 1.8 µm. It does not grow at 37° C, and grows very slowly at 28° C. After eight days at 28° C, colonies on BHI agar were 0.4 - 1.5 mm in diameter, hyaline, butyrous in texture, with margins entire. Glucose and lactose are not fermented. There was no identity within the BioLog Gram-negative library but all isolates of the formed a similarity grouping, suggesting that it is one species.

Plans: This study has been completed. No further work along these lines is planned.

AERIAL SPRAY EXPERT SYSTEM (ASPEX) FOR MOSQUITO CONTROL

G.A. Mount, T.L. Biery, D.G. Haile, and E. Daniels

Objective: To develop an expert system to reduce undesirable environmental contamination and horizontal transport from aerial spray applications for mosquito and other biting fly control. ASPEX is designed to assist in the training of aerial spray personnel.

Methods: With the support of T. L. Biery, Aerial Spray Branch, U. S. Air Force Reserve, Vienna, OH, the DoD Legacy Resource Management program provided funds to ARS for the development of an expert system for training of aerial spray personnel with the main goal of reducing environmental contamination while still achieving satisfactory mosquito control. The participants in the development of ASPEX have met several times during the past 14 months to develop a framework for the software program. A working version of ASPEX has been programmed in Visual Basic for demonstration and review by project personnel and potential users.

One aspect of the expert system development was a comprehensive literature review of ULV aerial spray. Topics of the review include application volume, adulticiding, larviciding, droplet size, and meteorology. The review discusses the efficacy of ULV aerial sprays against many important pest and vector species of mosquitoes in a wide range of locations and habitats in the U.S., Asia, Africa, and the Americas.

Results: Nine conclusions were drawn from this review. (1) ULV applications are as effective for mosquito control as highly-diluted, water-based sprays. (2) More acres can be sprayed per aircraft load with the ULV method than with dilute sprays. (3) High altitude ULV sprays could be used in emergencies if wind speed and direction data are available to accurately place the spray. (4) Successful adult mosquito control can be achieved in dense foliage or open housing with ULV aerial sprays, but doses of insecticide must be increased. (5) ULV aerial application of mosquito larvicides can be used successfully in large areas. (6) The optimum droplet size for adult mosquito control is 5-25 μm volume median diameter (VMD). (7) For mosquito adulticiding, near optimum atomization of ULV sprays is achieved with flat-fan nozzles oriented straight down or slightly forward for high-speed aircraft ($\geq$150 mph) or rotary atomizers on slow-speed aircraft (<150 mph). (8) Optimum atomization minimizes paint spotting. (9) Maximum adult mosquito control is achieved just after sunrise and just before sunset with 2-10 mph crosswinds.

Plans: The working version of ASPEX, including review of literature and all literature, is available on CD-ROM for use on IBM-compatible PCs.

SIMULATION OF MANAGEMENT STRATEGIES FOR THE BLACKLEGGED TICK, *Ixodes scapularis*, AND THE LYME DISEASE SPIROCHETE, *Borrelia burgdorferi*

G.A. Mount, D.G. Haile, and E. Daniels

Objective: To model management technologies for incorporation into LYMESIM and to simulate the effectiveness of technologies alone and in various combinations for both short and long-term management of populations of blacklegged ticks and the Lyme disease spirochete.

Methods: Tick management technologies reported in the literature include area-wide acaricide applications, acaricide-treated cotton for self-treatment of white-footed mice, acaricide self-treatment of white-tailed deer, vegetation reduction, and white-tailed deer density reduction. These management technologies were incorporated into our computer model of blacklegged tick population dynamics and Lyme disease agent transmission, LYMESIM. Modeling of technologies was based on available data from the literature and was designed to quantify their effects on tick populations. Some approximations of factors and relationships were made to include the technologies in LYMESIM. Because the model assumes a uniform distribution of ticks and hosts, technologies were developed without regard for the clumped distribution of *I. scapularis* or for possible reinfestation by movement of infested hosts from unmanaged into managed areas. Thus, simulation results with LYMESIM represent mean tick population levels and mean densities of ticks infected with *B. burgdorferi*. The model simulates tick and host populations on a per ha basis rather than for any specific size of target area. Simulation results can represent any size ecosystem; however, results of long-term simulations may not be realistic unless they represent an area that is isolated or large enough to exceed the home range of the resident white-tail deer population.

Results: Results showed that area-wide acaricide, vegetation reduction, or a combination of these technologies would be useful for short-term, seasonal management of ticks and disease in small recreational or residential sites. Acaricide self-treatment of deer appears to be the most cost-effective technology for use in long-term management programs in large areas where deer are not hunted. Simulation results also suggested that deer density reduction should be considered as a management strategy component in areas where deer are hunted. Integrated management strategies are presented that could be used in pilot tests and/or operational tick and tick-borne disease programs.

Plans: This computer simulation study was completed and copies of LYMESIM software for operation on IBM-compatible PCs and reprints are available upon request from the authors.

SIMULATION OF BLACKLEGGED TICK, *Ixodes scapularis,* POPULATION DYNAMICS AND TRANSMISSION OF THE LYME DISEASE SPIROCHETE, *Borrelia burgdorferi*

G.A. Mount, D.G. Haile, and E. Daniels

Objective: To synthesize new knowledge on the population dynamics of the blacklegged tick and its transmission of the Lyme disease spirochete.

Methods: Our approach to modeling these biological systems included the design of interactive simulation software and features a dynamic life table for *I. scapularis* with weekly age classes and time steps. Weekly rates of development, survival, fecundity, and host finding are based on weather or other environmental variables and vary with time. The model (LYMESIM) is deterministic and assumes that ticks and hosts are uniformly distributed. We also assumed no inherent biological variation in *I. scapularis* populations with respect to relationships used in LYMESIM. Epidemiological parameters including host infectivity, tick infectivity, transovarial transmission, and transstadial transmission are included in LYMESIM to simulate transmission of the Lyme disease spirochete between vector ticks and vertebrate hosts.

Results: Validity of LYMESIM was established by comparing simulated and observed populations of immature *I. scapularis* on white-footed mice, *Peromyscus leucopus,* at two locations in Massachusetts. Validity was also indicated by comparisons of simulated and observed seasonality of blacklegged ticks in New York, Massachusetts, Florida, and Oklahoma-Arkansas. Further model validity was shown by correlation between simulated and observed numbers of immature ticks engorging on white-footed mice at three sites in Massachusetts. The model produced acceptable values for initial population growth rate, generation time, and 20-yr population density when historical weather files for 16 locations in eastern North America were used. Realistic rates of infection in ticks were produced for locations in the northeastern and north central U.S. LYMESIM was used to study the effect of white-footed mouse and white-tailed deer, *Odocoileus virginianus*, densities on tick density and infection rates. The model was also used to estimate tick density thresholds for maintenance of *B. burgdorferi.*

Plans: This computer simulation study was completed and copies of LYMESIM software for operation on IBM-compatible PCs and reprints are available upon request from the authors.

SIMULATION OF AREA-WIDE INTEGRATED MANAGEMENT STRATEGIES FOR THE LONE STAR TICK, *Amblyomma americanum*

G.A. Mount, D.G. Haile, and E. Daniels

Objective: To model management technologies for incorporation into LSTSIM and to simulate the effectiveness of technologies alone and in various combinations for both short and long-term management of populations of lone star ticks. Evidence is accumulating that *A. americanum* and white-tailed deer are natural vector and reservoir host, respectively, of *Ehrlichia chaffeensis* Anderson, Dawson, and Wilson, the causative agent of human monocytic ehrlichiosis.

Methods: Tick management technologies reported in the literature include area-wide acaricide applications, acaricide self-treatment of white-tailed deer, vegetation reduction, and white-tailed deer density reduction. These management technologies were incorporated into our computer model of lone star tick population dynamics, LSTSIM. Modeling of technologies was based on available data from the literature and was designed to quantify their effects on tick populations. Some approximations of factors and relationships were made to include the technologies in LSTSIM. Because the model assumes a uniform distribution of ticks and hosts, technologies were developed without regard for the clumped distribution of *A. americanum* or for possible reinfestation by movement of infested hosts from unmanaged into managed areas. Thus, simulation results with LSTSIM represent mean tick population levels and mean densities of ticks. The model simulates tick and host populations on a per ha basis rather than for any specific size of target area. Simulation results can represent any size ecosystem; however, results of long-term simulations may not be realistic unless they represent an area that is isolated or large enough to exceed the home range of the resident white-tail deer population.

Results: Results showed that area-wide acaricide, vegetation reduction, or a combination of these technologies would be useful for short-term, seasonal management of ticks and disease in small recreational or residential sites. Acaricide self-treatment of deer appears to be the most cost-effective technology for use in long-term management programs in large areas where deer are not hunted. Simulation results also suggested that deer density reduction should be considered as a management strategy component in areas where deer are hunted. Integrated management strategies are presented that could be used in pilot tests and/or operational tick and tick-borne disease programs.

Plans: This computer simulation study was completed and copies of LSTSIM software for operation on IBM-compatible PCs and reprints are available upon request from the authors.

TAXONOMIC ANALYSIS OF THE FIVE MEMBERS OF THE *Anopheles quadrimaculatus* COMPLEX

J.F. Reinert, P.E. Kaiser, K. Kangas, J.A. Seawright, and M. Falkner

Objective: To analyze the morphological characteristics and produce a taxonomic key for the five members of the *Anopheles quadrimaculatus* complex. Historically, this species was the principal vector of malaria and an important pest in the eastern half of the United States. This current study was conducted to provide a taxonomic key that would be of value to people involved in mosquito control programs.

Methods: Females of the five species in the complex were collected from a number of field sites, were fed a blood meal, and allowed to oviposit in vials. Identification to species of these females initially was confirmed by starch gel electrophoresis and each isofemale brood was separately reared. The associated pupal and fourth instar larval exuviae for each reared adult were saved for morphological examination. Some fourth instar larvae of each isofemale brood were also preserved. Adults were mounted on points attached to pins and the immature exuviae and whole larvae were mounted in Canada balsam on microscope slides. The genitalia of adult males were dissected and mounted. All specimens were then studied using established morphological taxonomic procedures.

Results: During the morphological taxonomic study, 139 isofemale broods were reared for the five cryptic species. This resulted in 5,284 mounted adults on pins, their associated pupal and larval exuviae mounted on slides, over 2,000 fourth instar larvae mounted on slides, and 199 male genitalia dissected and mounted on slides. Final examination and evaluation have been completed for females, male genitalia, and pupae. Final analysis of the larvae is underway. Descriptions and keys for females, male genitalia, and pupae, and written sections on larval and pupal chaetotaxy, geographic distribution and bionomics are finished. Preliminary illustrations of pertinent stages and structures have been drawn. The literature review for the complex is complete and includes over 1,600 citations. Results of the study indicate that females, pupae, and larvae of the five species can be separated by morphological characters, four of the species can be separated by the male genitalia. One paper has been published concerning unusual features of the pupae and two papers have been completed and are ready for peer review, viz. a bibliography of *An. quadrimaculatus* sensu lato, and a report of atypical habitats for larvae of *An. quadrimaculatus* species A.

Plans: This phase of the study is nearly completed. The final results will be published in the form of a monograph during the next year.

NON-BITING MUTANTS OF *Aedes aegypti*

J.A. Seawright, M. Falkner, and K. Kangas

Objective: To induce and isolate strains of *Aedes aegypti* that cannot bite. Non-biting mutants could be very useful as a means to disseminate a very effective microsporidean pathogen of *A. aegypti*.

Methods: Initiation of this project consisted of three components. (1) Recessive mutants were rescued from an existing colony and two pure-breeding strains were established. The mutants were white-eye (*we*) and red-eye (*re*) on chromosome I, spot abdomen (*s*) on chromosome II, and black tarsi (*blt*) on chromosome III. A three generation cross scheme and subsequent inbreeding was carried out to establish strains that were homozygous, i.e. *we s blt*, and *re s blt*. A cross scheme was also carried out to establish a strain homozygous for *re we s blt*; *we* is epistatic over *re*, and this required progeny testing over two generations. (2) A concentration-sterility relationship was determined for the mutagen, ethyl-methane sulphonate (EMS). Males (<20 hours old) were fed (ad lib) EMS in 10% sucrose solution, and the resultant sterility was measured. (3) Defibrinated bovine blood (heated to 40° C) was fed to female *A. aegypti* on cotton pads to determine whether they could develop eggs. Detection of dominant non-biting mutants was assessed directly by observing whether the F_1 females were capable of taking a blood meal. Detection of recessive non-biting mutants was done by using the mutant markers in a three-generation cross scheme designed to track portions of the EMS-treated chromosomes. The genetic map of *A. aegypti* is about 150 map units, and the mutant markers enabled us to assay effectively about 60 map units for non-biting, recessive mutants induced by EMS.

Results: Vigorous mutant strains were established for use in the mutant screen. The strain that is homozygous for *we* and *re* will be used to search for radiation-induced, balancer-inversion strains, which will be useful for future needs whenever maximum inbreeding of chromosome I (which contains the sex-determining element) is required. We determined that a concentration of 4 microliters of EMS/milliliter of 10% sucrose solution caused about 50% sterility, and this concentration was selected as a treatment for the induction of non-biting mutants. We also determined that females of *A. aegypti* can develop eggs after a blood meal of bovine blood presented on a cotton pad. In screening for non-biting mutants, we have yet to achieve success in terms of isolation of suitable mutants. We have isolated numerous non-biting females, but none of these non-biting types were due to the kind of simple Mendelian trait that we are seeking.

Plans: This project is continuing.

SUGAR ACQUISITION DURING THE DEVELOPMENT OF MICROSPORIDIAN (MICROSPORA: NOSEMATIDAE) SPORES

A.H. Undeen and L.F. Solter[1]

Objective: To determine if the increased density of microsporidian (Protozoa) spores on Ludox density gracients results from the accumulation of sugar and the age of the spores affected.

Methods: *Vairimorpha necatrix* spores were obtained from the Illinois Natural History Survey, Champaign, IL and *Nosema algerae* spores and *Helicoverpa zea* were produced at USDA/CMAVE in Gainesville, FL. Larval *H. zea* were starved overnight, fed ca. 2 x 10^6 spores from a bacteriological loop, placed on artificial diet and reared at 27±1°C. Daily, from 3 to 8 days after inoculation, larva were individually homogenized, filtered through cotton to remove host tissues, placed on Ludox density gradients and centrifuged for 30 minutes at 16,300 *g*. Density of the developing spores was calculated from the position of the spores in the gradient. Bands of spores were withdrawn from the gradients, cleaned by two 40-ml rinses in water, concentrated in a small volume and counted in a hemocytometer. Refringence was estimated by measuring the optical density of spore suspensions in a spectrophotometer (625 nm). The length and width of spores from the various bands were measured with a Vickers A.E.I. image-splitting micrometer and volumes caculated as a prolate spheroid. Spore weight is the product of density and volume. Viability of *V. necatrix* spores was determined by infecting *H. zea* larvae and *N. algerae* by germination in pH 9.5 buffered, 0.1 M NaCl. Samples of spores from each band were homogenized by shaking with glass beads. Total sugar in the supernatants of all samples was measured by the hot anthrone method and reducing sugars by the Nelson's test.

Results: *Vairimorpha necatrix* spores, 5 to 8 days after inoculation, usually formed 4 bands in the gradients. Bands 1 and 2 were small, with buoyant densities of 1.072 and 1.110 respectively. Bands 3 (1.150 g/ml) and 4 (1.198 g/ml) formed later, were more consistent in location and contained the bulk of the spores. The bands were found in approximately the same position regardless of the time after infection. Optical density was 0.172, 0.241, and 0.368 for the stages found in Bands 2, 3 and 4 respectively. Spores from Band 4 were significantly larger than spores in Bands 2 and 3. Spores of *V. necatrix* from Bands 2, 3 and 4 infected 5%, 11% and 95% of the test larvae respectively. The concentration of total sugar was significantly higher in Band 3 than in Band 4 spores (Table 1). Only trace amounts of sugar were found in spores from Bands 1 and 2. Sugar accounted for more than 60% of the increased spore weight from Bands 2 to 3 and Bands 3 to 4 (Table 1), the remainder is probably water. Similar results were obtained with spores of *N. algerae*, in which sugar accounted for more than 80% of weight gain demonstrating that sugars are acquired rapidly as the last act of sporulation. This permits separation of mature from immature spores for increased accuracy in bioassays.

Plans: Ultrastructural studies will correlate physical development of the spores with sugar acquisition.

[1]Center for Economic Entomology, Illinois Natural History Survey, 607 E. Peabody, Champaign, Illinois 61820

EGG PRODUCTION BY *STRELKOVIMERMIS SPICULATUS* (NEMATODA: MERMITHIDAE)

A.H. Undeen, S.E. White and T. Fukuda

Objective: To determine the timing of oviposition and production of *Strelkovimermis spiculatus* (Nematoda) eggs in three substrates: damp sand, saturated sand and water and to assess the effect of male presence on oviposition.

Methods: *Strelkovimermis spiculatus* postparasitic stages and adults are maintained in damp sand at 23±1°C, where they molt, mate and deposit their eggs. Cultures were flooded with water to obtain preparasites which were then presented to second instar *Aedes aegypti* at a rate of 4 preparasites per mosquito larva. Infected mosquito larvae were reared to the fourth instar, at which time the pre-adult worms emerged. One or 2 days after emergence, 10 female mermithids were placed in 100 ml dessert cups containing either 50 ml deionized water or 40 ml deionized water plus 35 cm^3 sand. Four mating situations were evaluated in both saturated sand and water: 1) males absent; 2) males continuously present; 3) males present for 7 days; 4) males present for 7 days, absent for 11 days and then returned.

Eggs were collected from the worms in deionized water and saturated sand at 3 to 4 day intervals, starting 7 days after the males and females were combined. Adult worms from the water sets were transferred to fresh cups after each collection. The saturated sand sets were washed twice with 30 ml aliquots of water to remove the eggs and adults. Dead females were recorded and removed and living adults were returned to the original sand. Eggs were harvested from the sets in damp sand by 2 washes in deionized water after the last count of the water and saturated sand sets were made. The number of eggs/female was calculated from counts of eggs in 3, 0.2 ml samples from each cup. Note was taken of whether females had completed oviposition.

Results: Overall mean survival (± SE) was 79.2 ± 3.1% (n = 24 sets of 10 adults each) at the end of the test. Oviposition did not occur in the absence of males. Egg production was best when males were continuously present ($6.4 \pm 0.9 \times 10^3$ eggs/female). Fewer eggs were produced when males were removed after 7 days ($2.8 \pm 0.2 \times 10^3$ eggs/female) and oviposition partially recovered after males were returned 11 days later ($4.4 \pm 0.5 \times 10^3$ eggs/female) (Table 1). The nematodes deposited substantially more eggs in sand ($6.4 \pm 0.9 \times 10^3$/female) than in water $1.9 \pm 0.3 \times 10^3$/female). Fifty percent of the eggs were produced by day 19.4 and 90% at day 26.4 after emergence of the pre-adult worms from the larval mosquitoes. Preparasites first appeared 24.5±1.5 days post emergence (20.5 days after adding the males or about 14 days after oviposition began) in the two saturated sand cultures but they never exceeded 10% of the number of eggs collected. When eggs were harvested from the damp sand at 34 days after emergence, 22.5 ± 4.3% of them hatched immediately. Oviposition was nearly complete by 35 days after the emergence of the adult worms from the mosquito larvae.

Plans: Similar studies will continue so as to provide the information necessary to predict where this mermithid might be capable of cycling through successive generations of mosquitoes and to develop a viable product that can be stored and shipped.

POSTHARVEST

AND

BIOREGULATION

CRIS - 6615-43000-007-00D -- Population Management of Insects to Protect Stored Products

CRIS - 6615-43000-008-00D -- Detection and Population Estimation of Stored Product Insects

THE EFFECTS OF JUVENOIDS ON EMBRYOGENESIS IN *PLODIA INTERPUNCTELLA & SITOPHILUS ORYZAE*

S. Dyby and D. Silhacek

Objective: To determine the physiological basis of ecdysteroid and juvenoid agonist toxicities during embryogenesis and early larval development of the Indianmeal moth and the rice weevil.

Methods: Freshly laid eggs were collected from a standardized laboratory culture of *Plodia interpunctella*, the Indian meal moth. The eggs were carefully aged following egg laying in order to isolate previously established developmental events that occur during embryogenesis. These events were monitored by time lapse photography, light microscopy, scanning and transmission electron microscopy, and fluorescent microscopy. Embryonic development in the presence and absence of the hormone agonists were compared in order to identify any lesion(s) that occur in response to a treatment. Similar studies were carried out with the rice weevil. However, with this insect, eggs were collected by placing gravid females onto cornstarch for finite time periods and then collecting the eggs by dissolving the starch. Embryonic development was then monitored in the presence and absence of hormone agonists as described earlier.

Results: Treatment of newly laid eggs of *P. interpunctella* with a juvenile hormone agonist (JH_{Ag}) causes midgut closure to be disrupted about 35 hours later (at 30°C). The embryo normally repositions itself within the egg at this time by undergoing specific bending movements; JH treatment interferes with these movements. We suggest that these abnormalities in embryonic movements are interrelated with the abnormalities of midgut closure and that the underlying defect may reside with the yolk cells. Some yolk cells are positioned on the splanchnic mesoderm before dorsal closure, others appear to move into the embryonic cavity during dorsal closure. Since yolk cells adhere to each other and are motile, their movement can exert considerable pull on the adjacent embryonic mesoderm and ectoderm, as well as the amnion This pull can effect the bending movements of the embryo and the final closure of the gut. In embryos treated with a JH_{Ag}, the yolk cells tend to stack up on the dorsal side of the embryo where they appear to present a considerable restraint to normal embryonic bending movements and midgut closure. Direct treatment of newly laid eggs of *Sitophilus oryzae* was accomplished by incubating the eggs in Halocarbon oil (wt. 700) containing the JH_{Ag}. The majority of *Sitophilus* embryos hatch at fenoxycarb levels of 1 ppm, a level that kills 95% of the *Plodia* embryos. Increasing fenoxycarb levels to 10 ppm prevents about 80% of the *Sitophilus* embryos from hatching. Some apparently normal prelarvae die while attempting to hatch, because they are unable to shed the embryonic cuticle. Defects in the tracheal system (e.g. sections of the lateral trunk are missing) are commonly observed in those that do hatch, so that survival beyond the 1st instar is unlikely. Light microscopy examination of the embryo has not yielded any definite evidence of abnormalities in the gut because of the opacity and different shape of the gut. When the ecdysteroid agonist (E_{Ag}), RH 5892, is applied to *Plodia* eggs, it is only effective when applied between 25 and 29 hrs after the eggs are laid. Treating eggs with low levels of E_{Ag} induces hypertrophy of the Malpighian tubules, prevents tanning and may cause abnormalities in eye formation. When early instar larvae are fed diet containing this E_{Ag}, they frequently fail to molt and if they do molt, the cuticle remains very sticky.

Plans: During the next year we will complete our studies on the effects of JH_{Ag} on embryonic development in *Sitophilus oryzae*. In addition, we will continue our investigations on the mode of action of the E_{Ag} to determine whether their effects can be incorporated into more effective protocols that can be used for the protection of stored commodities from insect damage.

ACTION OF A JUVENILE HORMONE MIMIC, FENOXYCARB, ON A MOTH CELL LINE

H. Oberlander and C.E. Leach

Objective: To determine whether an established cell line derived from imaginal discs of *Plodia interpunctella* can be utilized for investigations of the mode of action of juvenile hormone.

Methods: The IAL-PID2 cell line was established from wing imaginal discs of the Indianmeal moth (J. Insect Physiol. 29:591. 1983). This cell line has been used intensively for studies on the action of 20-hydroxyecdysone, with particular emphasis on ecdysteroid effects on proliferation and on processes related to chitin synthesis. The cells are maintained in tissue culture flasks in antibiotic-free Grace's medium supplemented with 10% heat-inactivated fetal bovine serum. Radio-labeled GlcNAc was added to the flasks to measure uptake of this chitin precursor by the cells, as measured by a liquid scintillation counter after solubilization of the cells. The cell number was assessed by direct counts of samples of the cultures using a Spencer bright line hemocytometer with phase microscopy.

Results: We demonstrated that both 20-hydroxyecdysone (1.0μM) and the non-steroidal ecdysteroid mimic, RH-5992 (0.005 μM), increased GlcNAc uptake and decreased proliferation by the PID2 cells. to test for juvenile hormone effects, the juvenile hormone, mimic, fenoxycarb (10μM), was added to the cultures simultaneously with the ecdysteroid or ecdysteroid agonist. Under these conditions fenoxycarb reduced ecdysteroid-stimulated GlcNAc uptake/cell when the cultures were examined after three days of continuous exposure to the hormones in culture. In addition proliferation of cells in the fenoxycarb treated cultures was reduced. Thus, in the case of GlcNAc uptake fenoxycarb was inhibiting an ecdysteroid-induced effect, while by inhibiting proliferation it was mimicking an ecdysteroid action on the cells.

Plans: The possible use of the PID2 cell line for investigating the action of juvenile hormone mimics will be evaluated further. Improvements in methodology are needed to reduce variability in the responses to fenoxycarb. Additional juvenile hormone mimics will be evaluated with this cell line assay.

ACTION OF ECDYSTEROID AND JUVENOID AGONISTS ON LARVAE OF *Plodia interpunctella*

H. Oberlander, D.L. Silhacek and S. Parisek

Objective: To determine the actions of two potent non-steroidal agonists, RH-5992 and RH-2485, and the juvenile hormone mimic, methoprene, on growth and development of *Plodia interpunctella.*

Methods: Indianmeal moth larvae were fed a cereal diet treated with RH-5992 or RH-2485 (provided courtesy of Robert Haas Co.) and methoprene, separately and in combination. The weight and developmental stage of the treated insects were monitored during a one month period.

Results: Larvae treated with methoprene (10 ppm) and RH-5992(≥5 ppm) or RH-2485(≥5 ppm) continued to grow , while those treated with RH-5992 or RH-2485 alone gained little weight. Moreover, methoprene delayed, but did not prevent RH-5992 or RH-2485 induced mortality. At low concentrations RH-2485 caused reduced emergence without reducing larval survival, suggesting a juvenile hormone-like effect. Thus, these experiments demonstrated antagonistic interactions between ecdysteroid and juvenoid agonists (Table 1).

TABLE 1. EFFECT OF RH-5992, RH-2485 AND METHOPRENE ON THE INDIANMEAL MOTH

PPM IN DIET					
RH-5992	RH-2485	METHOPRENE	Day 0 WEIGHT(mg)	Day 3 WEIGHT(mg)	%MORTALITY
0	0	0	3.09	15.01	5
0	0	10	3.18	15.83	32
5	0	0	3.32	7.93	70
5	0	10	3.66	12.45	66
0	5	0	3.08	7.87	50
0	5	10	2.81	11.70	76

Plans: Tests will continue to evaluate non-steroidal ecdysteroid mimics for effectiveness against stored product insects.

ISOLATION AND PARTIAL SEQUENCING OF A cDNA CLONE FOR VITELLOGENIN FROM THE INDIANMEAL MOTH, *Plodia interpunctella*

P.D. Shirk

Objective: To obtain the gene for vitellogenin protein that is produced in the fat body of the female Indianmeal moth, *Plodia interpunctella*

Methods: Fat body mRNA from late pharate adult females was used to construct a cDNA expression library in lambda Zap II. The cDNA library was screened with antigen selected antiserum to YP1 a subunit of vitellin. After three rounds of selection using the YP1 anitserum, the Bluescript II phagemid was excised from the lambda carrier. The DNA insert was partially sequenced from both ends. The cDNA sequence was used to search the GenBank to identify homologies with other known genes and proteins.

Results: Three cDNA clones that reacted with the YP1 antiserum were isolated and subjected to DNA sequencing. These independent clones contained an approximately 2 kilobase sequence that produced an predicted amino acid sequence identical to the amino acid sequence of the amino terminus of the YP1 subunit purified from yolk. The YP1 DNA sequences had up to 60% identity with vitellogenin from *Bombyx mori*.

Plans: The gene for YP1 will be isolated from a genomic DNA library and the regulatory DNA sequences will be identified. The regulatory sequences that result in fat body specific expression will be used to develop a germ line transformation method for genetic production of conditionally sensitive lethal insects for use in pest insect control programs.

ISOLATION AND SEQUENCING OF A cDNA CLONE FOR AN α-CRYSTALLIN PROTEIN COGNATE IN GERM CELLS OF THE INDIANMEAL MOTH

P.D. Shirk and R. Broza

Objective: To obtain the gene for a major germ cell protein, *ac25,* that was previously isolated and characterized by this laboratory.

Methods: Ovarian mRNA from late pharate adult females was used to construct a cDNA expression library in lambda Zap II. The cDNA library was screened with antigen selected antiserum to an α-crystallin protein. After three rounds of selection using the *ac25* antiserum, the Bluescript II phagemid was excised from the lambda carrier. The DNA insert was sequenced in both directions. The cDNA sequence and the predicted amino acid sequence were used to search the GenBank to identify homologies with other known genes and proteins.

Results: Four cDNA clones that reacted with the α-crystallin antiserum were isolated and subjected to DNA sequencing. These independent clones contained a 796 base pair sequence. The sequence contained a 185 codon open reading frame and amino acids 71 - 100 were identical to the amino acid sequence of an internal fragment previously identified. The predicted amino acid sequence had 60% similarity with the embryonic lethal, el(2)13.1, found in *Drosophila melanogaster* and 50% similarity with α-crystallin proteins found in the lens of vertebrate eyes. The sequence had much less similarity with the small heat shock proteins from *D. melanogaster.* The DNA sequence for *ac25* also showed considerable similarity with *D. melanogaster* el(2)13.1. However, the small heat shock protein genes of *D. melanogaster* were more related to ac25 than were the genes for the α-crystallin proteins. This suggests that the similarity observed in the amino acid sequences between the α-crystallin proteins and *ac25* was convergent evolution. We have shown previously that *ac25* is a chaperon for the follicular epithelium yolk protein. The similarity in structure between these two groups of proteins may indicate a convergence on a related binding domain.

Plans: The gene for *ac25* will be isolated from a genomic DNA library and the regulatory DNA sequences will be identified. The regulatory sequences that result in germ cell specific expression will be used to develop a germ line transformation method for genetic production of sterile insects for use in sterile insect release programs.

ISOLATION AND SEQUENCING OF A cDNA CLONE FOR YP2: A SUBUNIT OF THE FOLLICULAR EPITHELIUM YOLK PROTEIN OF THE INDIANMEAL MOTH, *Plodia interpunctella*

P.D. Shirk and R. Broza

Objective: To obtain the gene for the yolk polypeptide 2 (YP2) subunit of the follicular epithelium yolk protein that was previously isolated and characterized by this laboratory.

Methods: Ovarian mRNA from late pharate adult females was used to construct a cDNA expression library in lambda Zap II. The cDNA library was screened with antigen selected antiserum to YP2. After three rounds of selection using the YP2 anitserum, the Bluescript II phagemid was excised from the lambda carrier. The DNA insert was sequenced in both directions. The cDNA sequence and the predicted amino acid sequence were used to search the GenBank to identify homologies with other known genes and proteins.

Results: Six cDNA clones that reacted with the YP2 antiserum were isolated and subjected to DNA sequencing. These independent clones contained a 1984 base pair sequence. The sequence contained a 618 codon open reading frame and amino acids 18 - 48 were identical to the amino acid sequence of the amino terminus of YP2 purified from yolk. The YP2 gene had 50% identity with the egg specific protein (ESP) from *Bombyx mori*. The most significant differences between YP2 and ESP were in the amino terminal region of the proteins. However, YP2 shared a consensus lipase sequence that has been identified in *B. mori* ESP and the three yolk polypeptides of *Drosophila melanogaster*.

Plans: The gene for YP2 will be isolated from a genomic DNA library and the regulatory DNA sequences will be identified. The regulatory sequences that result in follicular epithelial cell specific expression will be used to develop a germ line transformation method for genetic production of sterile insects for use in sterile insect release programs.

POTENTIAL USE OF JUVENOIDS FOR PROTECTING STORED PRODUCTS FROM INSECT PESTS

D. Silhacek and C. Murphy

Objective: To determine if treatments with juvenoid agonists, ecdysteroid agonists or chitin synthesis inhibitors can effectively protect commodities from insect damage during storage.

Methods: In our studies during the past year, the rice weevil, Sitophilus oryzae was studied. They were reared on whole wheat at 26° C and 70% RH under a 16L:8D light cycle. The test compounds, fenoxycarb and pyriproxyfen (juvenoid agonists, JH_{Ag}), RH-5849 and RH-5992 (ecdysteroid agonists, E_{Ag}) and chlorfluazuron and diflubenzuron (chitin synthesis inhibitors, CSIs) were applied topically to adult weevils; their subsequent fecundity was observed over the next three to four weeks by placing them on corn starch or pasta to facilitate determination of the number of eggs laid.

Results: When mated females were placed on a cornstarch diet treated with fenoxycarb or pyriproxyfen, the number of offspring depended upon the dose of the JH_{Ag}. For example, when the diet contained 5 ppm fenoxycarb, all eggs laid in the media failed to hatch; for comparison, 80 % of the eggs laid on untreated cornstarch hatched. The effectiveness of fenoxycarb was increased up to 100-fold by the addition of the synergist, piperonyl butoxide, (1:10, w/w). Higher ratios of synergist further increased the effectiveness of fenoxycarb. A similar effect was observed with pyriproxyfen. When adults were topically treated with fenoxycarb or pyriproxyfen (one µl of ≥10 ppm in acetone) and then placed on pasta to lay eggs, all eggs failed to hatch; in controls treated with acetone alone, all of the eggs laid, hatched. The effectiveness of the JH_{Ag} could be increased about 20-fold by the addition of piperonyl butoxide (1:10, w/w). The number of eggs laid by females treated with a JH_{Ag} increased in response to increases in the dose of JH_{Ag} administered. This stimulation of egg production by females weevils was observed when JH_{Ag} was placed in the diet or was topically applied. Even though the JH_{Ag} stimulated additional egg production, reproduction was severely impaired because of JH_{Ag}-induced abnormalities in embryonic development. Treating males with a JH_{Ag} prior to mating had no effect on the number or hatchability of eggs laid by the female partner. We have conducted preliminary tests on the effects E_{Ag} and CSIs on the reproduction of the rice weevil. We treated mated females by transferring them to cornstarch diets containing different levels of chlorfluazuron. We found that the number of eggs produced per female was unaffected. However, the number of offspring produced was reduced in a dose-dependent response. When the level of chlorfluazuron was ≥ 1 ppm in the diet, none of the eggs hatched, indicating a lethal impairment in embryonic development. Diflubenzuron and the E_{Ag} were relatively ineffective when assayed by this procedure.

Plans: Our current investigations have provided a number of interesting leads that could be developed into effective protocols for protecting stored commodities. During the next year, we will examine the applicability and practicality of this technology when used to protect commodities when stored in bulk and in packages following processing.

EFFECTS OF HEAT TREATMENT ON ACOUSTICAL DETECTION OF LARVAE IN SAMPLES OF STORED GRAIN

R. W. Mankin, D. K. Weaver and D. Shuman

Objectives: To determine whether exposing stored product insect larvae to short bursts of heat can increase the likelihood or frequency of detectable feeding or movement sounds. Increases in the level of sound production will improve the reliability of acoustical detection technology.

Methods: An automated acoustical monitoring system was used to detect and time the sounds produced by 4th-instar *Sitophilus oryzae* larvae infesting 0.2 -kg samples of grain. Samples of infested or clean grain were monitored for two h, transferred to a moisture balance, exposed under a heat lamp for a specified duration, and then monitored for two h after treatment. About 250 adults subsequently emerged from each infested sample, on average. The first set of treatments tested was an exposure of 4 min at a heat setting which raised the temperature at the center of the grain mass to 30°C (30°C-Heat), or the identical treatment with no heat (No-Heat).

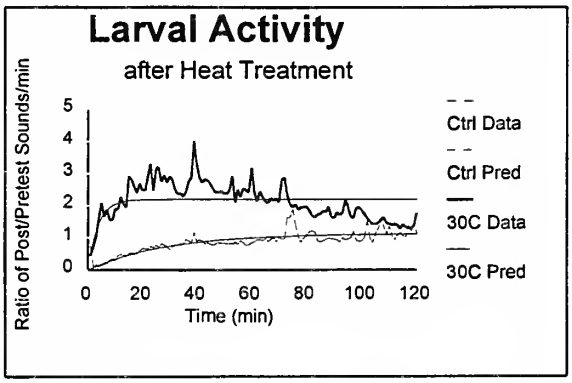
Results and Plans: We compared the activity levels in 1-min intervals after treatment to the mean activity level (per minute) during the 45 min before treatment. A regression was fitted to the results:

$$\text{Activity} = B0(1-e^{-B1t})$$

The regression coefficients are presented in the Table and the data are plotted in the figure.

Relative Activity of Post- to Pre-Heat Tests of *S. oryzae*-Infested Grain

Parameter	30°-Heat		No-Heat
B0			
Estimate	2.204	1.14	
Std. Error	0.052	0.04	
Lower 95%	2.10		1.06
Upper 95%	2.31		1.21
B1			
Estimate	32.62		4.24
Std. Error	8.55		0.45
Lower 95%	15.68		3.35
Upper 95%	49.56		5.14



As expected, B0 is about 1 for No-Heat, i.e., there was little net effect of handling. In both cases, B1 was statistically significant, i. e., handling temporarily reduced the activity level. However, within 20 min after the heat treatment, the activity level was about double the pretreatment level (B0 = 2.2). These results indicate that heat treatment has potential for increasing the reliability of acoustical insect detection techniques, and testing will be continued at progressively higher heat levels until the effect declines.

SERIAL MULTIPLEXING ADDRESSABLE REGISTER TRANSMISSION SYSTEM (SMARTS)

D. Shuman

Objective: To develop an economically attractive data transmission facility suitable for data collection by a single PC type computer from a large number of remote sensor locations distributed throughout a large grain storage facility. This system could be used in conjunction with the EGPIC System and with the acoustic sensors mounted on cables for monitoring insect infestation.

Methods: The original design of the SMARTS facility was modified to halve the amount of wire needed by time sharing single twisted wire pairs for transmitting and receiving. It is capable of automatically sequencing through any programmed order of remote locations or manually selecting any individual remote location. SMARTS can be implemented with different numbers of multiplexing levels with different numbers of multiplexer inputs to minimize the hardware costs for individual applications with a maximum addressing capacity of more than one million one-byte digital registers. Using RS-422 protocol, the computer could be located many miles away from the furthest register.

Results: The new SMARTS design is operational in the laboratory. A SMARTS patent application has been submitted to the U.S. Patent Office.

Plans: The SMARTS software will be expanded to provide a user interface. The system will be tested using long transmission lines. In collaboration with Dr. D. Hagstrum of the ARS laboratory in Manhattan, KS, a large-scale version of the system will be constructed for use with acoustic sensors as part of a stored-grain infestation monitoring system. Custom electronics will be designed to allow the insect sound data gathered by the acoustic sensors to be locally digitized and accumulated for transmission to a computer via the SMARTS data transmission facility. The system will be field tested in a grain elevator.

ELECTRONIC GRAIN PROBE INSECT COUNTER (EGPIC)

D. Shuman and D. Weaver

Objective: To develop and evaluate a grain probe that counts insects electronically as they fall through. Commercial grain probe traps presently available are effective in monitoring low infestations levels in stored grain. However, ascertaining the trap insect count is labor intensive involving removal of the trap from the grain, visual inspection of the contents, and re-insertion of the trap. There is also the tradeoff between trap inspection frequency, timely discernment of infestation, and cost. EGPIC would eliminate these concerns by providing remote real-time data.

Methods: A commercial grain probe trap was modified by incorporating a custom sensor head with infrared electronics to count insects as they fall through. A versatile modular design allows for multiple system implementations to address different applications. For example, a system suitable for small storage facilities was constructed that connects the grain probe to a control box that is mounted on the outside of a grain bin. The control box has an LED insect count display, a power interrupt indicator, and self-test and reset controls. A computer-based system was developed that creates a data base of the time-stamped insect counts to be used for trend analysis, input to an expert management system, and automated control measures. It uses the printer port to interface with up to 8 probes (an installed digital I/O computer board could interface with up to 95 probes) for instantaneous (interrupt driven) probe activity updates. Another version, suitable for large storage facilities with thousands of probes miles away from a central computer, stores the insect count of each probe in its own digital register that can be polled through the serial port of the computer via a transmission network (SMARTS) designed for this purpose.

Results: Laboratory testing with infested grain showed >95% accuracy for the full range of pertinent insect species sizes. Field tests in a flat storage of corn in Wisconsin found that EGPIC overestimated the actual numbers of insects passing through the probes. Grain particles and dust that passed by the sensors, and the movement of minute insects and mites back and forth over the sensors, contributed to increased counts. Additionally, beam paths became obscured with accumulated dust as sampling time progressed. Nevertheless, regression analysis revealed that EGPIC counts could reliably predict (R^2=0.897) numbers of insects entering probes across a range of insect densities. Use of a different probe body with upward slanted holes resulted in fewer grain particles entering the probe and being counted. The sensor head has been redesigned to reduce the possibility of insects crawling and dust accumulating in the vicinity of the infrared beam. A patent is pending on the EGPIC system.

Plans: Upon completion of laboratory tests with the redesigned sensor head, the EGPIC system will be field tested in a Williston, FL, commercial wheat bin. Research will be conducted on the interpretation and utilization of the realtime data available with the EGPIC system. Enhancements of EGPIC that allow for species differentiation will be investigated.

ACOUSTIC LOCATION FINGERPRINTING INSECT DETECTOR (ALFID)

D. Shuman, D. Weaver and R. Mankin

Objective: To develop and evaluate an economically feasible inspection method to automatically identify the number of live insects, including internal-feeding larvae, in a grain sample. Present visual inspection methods can only detect adults because the larvae develop inside grain kernels.

Methods: An internal-feeding larva is normally not visible, but its presence can be revealed by detection of sounds it produces as it feeds within a grain kernel. Quantification of infestation by its correlation with total acoustic activity is not sufficiently accurate for sample grading. ALFID quantifies infestation by ascertaining the number of acoustic source locations in a grain sample. The system incorporates an array of acoustic sensors mounted in a grain sample container. A fingerprint related to the location of a particular sound's source can be produced by identifying the different sensors in the array that receive the sound, and the time intervals between those receptions. Multiple sounds originating from the same location, as determined by fingerprint matches, indicates the presence of an insect at that location.

Results: The prototype of the ALFID system that demonstrated feasibility of the operational concept for quantifying heretofore generally undetectable infestations of internal-feeding larvae as well as adult beetles was redesigned in an effort to improve its performance. The new grain container is a tube with two opposing arrays of 8 piezoelectric
microphones along its length. A CRADA has been established with DRT, Inc. to concurrently investigate applying a new acoustic sensor technology using fluidic amplification to reduce the electronic noise problems associated with detecting extremely low level larval sounds in the ALFID system. Acoustic signals from the sensor arrays are acquired by a customized A/D board in a PC computer and processed using cross-correlation and other time domain analyses to extract the time intervals (relative delays) between pairs of sensor output signals and reduce data artifacts by culling. Each sound's fingerprint, composed of its pattern of time intervals across all sensor pairs, is compared with all other sounds' fingerprints using a custom designed clustering and matching algorithm. Initial tests of the new system showed it to be 100% accurate in scoring grain samples with zero or one insects as compared to 90% and 70% respectively for the original prototype. In tests simulating two insects in a grain sample, the new system was 100% accurate when the insects were greater than two sensor spacings apart and 70% accurate when the insects were one sensor spacing apart. The original prototype could not distinguish between two insects less than 1.75 sensor spacings apart. Operation of the system in a noisy field environment is addressed by the use of acquisition-inhibiting ambient noise detectors mounted on the outside of the grain container which is then housed in a multi-layered sound attenuation box. The sound attenuation box can be rotated to different positions to facilitate the filling and emptying of the grain container without its being removed from the box and initial testing showed an average sound attenuation of 77 dB over the pertinent frequency range (1-10 kHz).

Plans: Upon completion of laboratory tests, ALFID will be field tested at a commercial elevator site. Future plans include the incorporation of fluidic sensor technology and neural network grouping of sounds. Behavioral research on factors affecting insect acoustic activity will be continued to increase the probability of detection during a sample test period. A patent on the ALFID system was issued in December, 1995.

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CENTER PUBLICATIONS LIST - OCTOBER 1995-SEPTEMBER 1996

Reprints may be requested by number

M-2953 HOGSETTE, J. A. and WASHINGTON, F. 1995 October. Quantitative Mass Production of *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae). J. Econ. Entomol. 88: 1238-1242.

M-2954 COCKBURN, A. F. and FRITZ, G. A. 1995 October. Isolation and Purification of Insect DNA (Book Chapter). Methods in Molecular Biology, Vol. 50, Chapter 2.

M-2955 HOGSETTE, J. A. 1995 January. Trapping Technology as a Sustainable Pest Technique. Proc. Of Department of Defense; 120-125.

M-2956 BARNARD, D. R., HARMS, R. H., and SLOAN, D. R. 1995 October. Influence of Nitrogen Phosphorus, and Calcium in Poultry Manure on Survival, Growth, and Reproduction in House Fly (Diptera: Muscidae). J. Econ. Entomol. 24: 1297-1301.

M-2957 GEDEN, C. J., LONG, S. J., RUTZ, D. A., and BECNEL, J. J. 1995 October. Nosema disease of the parasitoid *Muscidifurax raptor* (Hymenoptera: Pteromalidae): Prevalence, Patterns of Transmission, Management, and Impact. Biological Control 5: 607-614.

M-2958 BARNARD, D. R. 1995 September. Extration of House Fly (Diptera: Muscidae) Larvae from Poultry Manure Using Berlese/Tullgren Funnels. Fla. Entomol. 78 (3): 541-543.

M-2959 GEDEN, C. J., RUTZ, D. A., and STEINKRAUS, D. C. 1995 October. Virulence of Different Isolates and Formulations of *Beauveria bassiana* for House Flies and the Parasitoid *Musidifurax raptor*. Biological Control 5: 615-321.

M-2960 PORTER, S. D. 1995 May. Potential Use of Pseudacteon Phorid Flies as Fire Ant Biocontrol Agents (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 95.

M-2961 VANDER MEER, R. K., LOFGREN, C. S., and WILLIAMS, D. F. 1990 May. Methods for the Control of Insects (Patent). U.S. Patent No. 4,921,696.

M-2962 VANDER MEER, R. K., LOFGREN, C. S., WILLIAMS, D. F., MEYERS, W. E., and LEWIS, D. H. 1994 November. Methods for the Control of Insects (Patent). U.S. Patent No. 5,364,618.

M-2963 VANDER MEER, R. K., WILLIAMS, D. F., and LOFGREN, C. S. 1995 January. Composicao Para Controlar Uma Populacao de Artropodes (Patent). U.S. Patent Foreign Filing No. PI 8404556-6.

M-2964 OI, D. H., VAIL, K. M., KLOTZ, J. H., and WILLIAMS, D. F. 1995 May. Responses of *Tapinoma melanoccephalum*, Argentine Ants, and Pharaoh Ants to a Boric Acid Bait (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 27.

M-2965 TENNANT DE ALONSO, L. E., and VANDER MEER, R. K. 1995 May. Source of Alate Alarm Pheromones in *Solenopsis invicta* (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 33.

M-2966 WILLIAMS, D. F., COLLINS, H., OI, D. H., LOCKLEY, T., and CALLOT, A. M. 1995 May. Studies of an Imported Fire Ant Population in Eastern Tennessee (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 62-69.

M-2967 VANDER MEER, R. K., HEHMAN, G. L., COCKBURN, A. F., and SEAWRIGHT, J. A. 1995 May. Research on the Molecular Biology of Fire Ants (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 90.

M-2968 OI, D. H., and WILLIAMS, D. F. 1995 May. Effect of Alternative Bait Formulations on Imported Fire Ants and Non-Target Ant Populations (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 171.

M-2969 VANDER MEER, R. K., and SEAWRIGHT, J. A. 1995 May. Development of Pheromone Enhanced Baits and other Technology Transfer Activities (Abstract). Proc. of the 1995 Fire Ant Conference, San Antonio, TX, May 2-4, 1995 p. 179.

M-2970 FOCKS, D. A., DANIELS, E., HAILE, D. G., and KEESLING, J. E. 1995 November. A Simulation Model of the Epidemiology of Urban Dengue Fever: Literature Analysis, Model Development, Preliminary Validation, and Samples of Simulation Results. Am. J. Trop. Med. Hyg. 53: 489-506.

M-2971 BARNES, K. C. and BRENNER, R. J. 1996 January. Quality of Housing and Allergy to Cockroaches in the Dominican Republic. Int. Arch. Allergy & Immunol. 109: 68-72.

M-2972 ALLAN, S. A. and KLINE, D. L. 1995 December. Evaluation of Organic Infusions and Synthetic Compounds Mediating Oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). J. Chem. Ecol. 21(11): 1847-1860.

M-2973 PERERA, O. P., MITCHELL, S. E., COCKBURN, A. F., and SEAWRIGHT, J. A. 1995 November. Variation in Mitochondrial and Ribosomal DNA of *Anopheles quadrimaculatus* Species A (Diptera: Culicidae) Across a Wide Geographic Range. Ann. Entomol. Soc. Am. 88 (6): 836-845.

M-2974 VAIL, K. M. and WILLIAMS, D. F. 1995 December. Pharaoh ant (Hymenoptera: Formicidae) Colony Development after Consumption of Pyriproxyfen Baits. J. Econ. Entomol. 88: 1695-1702.

M-2975 KOEHLER, P. G. and MOYE, A. H. 1995 December. Airborne Insecticide Residues after Broadcast Application for Cat Flea (Siphonaptera: Pulicidae) Control. J. Econ. Entomol. 88: 1684-1689.

M-2976 KLOTZ, J. H. and MOSS, J. I. 1996 January. Oral Toxicity of a Boric Acid-Sucrose Water Bait to Florida Carpenter Ants (Hymenoptera: Formicidae). J. Entomol. Sci. 31: 9-12.

M-2977 BRIANO, J. A., PATTERSON, R. S., and CORDO, H. 1995 October. Relationship Between Colony Size of *Solenopsis richteri* (Hymenoptera: Formicidae) and Infection with *Theolhania solenopsae* (Microspordia: Thelohaniidae) in Argentina. J. Econ. Entomol. 88: 1233-1237.

M-2978 BRIANO, J. A., PATTERSON, R. S., and CORDO, H. 1995 October. Long-Term Field Studies of Black Imported Fire Ant, Solenopsis richteri (Hymenoptera: Formicidae), Infested with the Pathogen Thelohania solenopsae (Microsporida: Thelohaniidae) in Argentina. Environ. Entomol. 24: 1328-1332.

M-2979 BRIANO, J. A., PATTERSON, R. S., and CORDO, H. 1995 October. Colony Movement of the Black Imported Fire Ant, Solenopsis richteri (Hymenoptera: Formicidae) in Argentina. Environ. Entomol. 24: 1131-1134.

M-2980 BALDWIN, J. L., FOIL, L. D., and HOGSETTE, J. A. 1996 January. Important Fly Pests of Louisiana Beef Cattle. LSU Extension Bulletin Pub. 2617 (5M).

M-2981 ZHAO, R., KLOTZ, J. H., MOSS, J. I., DAVIS, JR. L. R., and PATTERSON, R. S. 1995 December. Laboratory evaluation of compounds for repellency against the cat flea (Abstract). Arthropod Management Tests; Entomology Society of America Vol. 20: 358-359.

M-2982 KLINE, D. L. and LEMIRE, G. F. 1995 December. Field Evaluation of Heat as an Added Attractant to Traps Baited with CO2 and Octenol for *Aedes taeniorhynchus*. J. Am. Mosq. Control Assoc. 11(4): 454-456.

M-2983 MOSER, B. A. 1995 December. Comparative Analysis of Microsporidia of Fire Ants, Solenopsis richteri and S. invicta. Univ. of Florida, Ph.D. dissertation, 125.

M-2984 VANDER MEER, R. K., SEAWRIGHT, J. A., AND BANKS, W. A. 1993 July. The Use of Repellents for Area Exclusion of Pest Ants (Abstract). Proc. 1st Intl. Conf. On Insect Pests in the Urban Environment, p. 494.

M-2985 VANDER MEER, R. K., and SEAWRIGHT, J. A. 1993 June. Fire Ant Orientation, Repellents and Bait Enhancement with Pheromones (Abstract). Proc. Of Fire Ant Conf., Charleston, SC, p. 67.

M-2986 VANDER MEER, R. K., and SEAWRIGHT, J. A. 1993 August. Repellent Activity of Interspecific Ant Alarm Pheromones Against *Solenopsis invicta*, the Red Import Fire Ant (Abstract). Proc. 10th Annual ISCE Meeting, p. 82.

M-2987 UNDEEN, A. H. and SOLTER, L. F. 1996 February. The Sugar Content and Density of Living and Dead Microsporidian (Protozoa: Microspora) Spores. J. Invertebr. Pathol. 67 (12): 80-91.

M-2988 BRIANO, J., JOUVENAZ, D., WOJCIK, D. P., CORDO, H., and PATTERSON, R. S. 1995 September. Protozoan and Fungal Diseases in *Solenopsis richteri* and *S. quinquecuspis* (Hymenoptera: Formicidae) in Buenos Aires Province, Argentina. Fla. Entomol. 78 (3): 531-537.

M-2989 MOSS, J. I. and SCOTT, J. G. 1996 February. Tetrodotoxin Protects German Cockroaches (Dictyoptera: Blattellidae) from Type I Pyrethroid and Carbamate Insecticide Poisoning. J. Econ. Entomol. 89: 51-55.

M-2990 VALLES, S. M. and YU, S. J. 1996 February. Detection and Biochemical Characterization of Insecticide Resistance in the German Cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 89 (1): 21-26.

M-2991 HOY, J. B., KOEHLER, P. G., and PATTERSON, R. S. 1996 March. A Microcomputer-Based System for Real-Time Analysis of Animal Movement. J. Neurosci. Methods 64: 157-161.

M-2992 VALLES, S. M. and YU, S. J. 1996 April. Tissue localization, induction, and developmental expression of cytochrome P450 monooxygenases in the German cockroach. Pesticide Biochem. Physiol.

M-2993 WILLIAMS, D. F. 1995 June. Controlling Fire Ants in the Amazon. Ciba Insect Control Newsletter 2:8.

M-2994 WILLIAMS, D. F. 1995 January. Controlling Fire Ants in the Amazon. Crop Protection News 1:6.

M-2995 SUITER, D. R., CARLSON, D. A., PATTERSON, R. S., and KOEHLER, P. G. 1996 April. Host-location Kairomone from *Periplaneta americana* (L.) for the Parasitoid *Aprostocetus hagenowii* (Ratzeburg). J. Chem. Ecol. 22: 637-651.

M-2996 VALLES, S. M., STRONG, C. A., and KOEHLER, P. G. 1996 April. Inter- and Intra-Instar Food Consumption in the German Cockroach, *Blattella germanica*. Entomol. Exp. Appl. 79: 171-178.

M-2997 XUE, R. D. and BARNARD, D. R. 1996 March. Human Host Avidity in *Aedes albopictus*: Influence of Mosquito Body Size, Age, Parity, and Time of Day. J. Amer. Mosq. Control Assoc. 12 (1): 58-63.

M-2998 LANDOLT, P. J. and QUILICI, S. 1996. Overview of Research on the Behavior of Fruit Flies. In: B. A. McPheron and G. J. Steck. Fruit Fly Pests. St. Lucie Press, Delray Beach, Florida, pp. 19-26.

M-2999 MANKIN, R. W., MALAVASI, A., and AQUINO, C. 1996. Acoustical Comparisons of Calling Songs from *Anastrepha* Species in Brazil. In: B. A. McPheron and G. J. Steck. Fruit Fly Pests. St. Lucie Press, Delray Beach, Florida, pp. 37-41.

M-3000 SIVINSKI, J. M. 1996. The Past and Potential of Biological Control. In: B. A. McPheron and G. J. Steck. Fruit Fly Pests. St. Lucie Press, Delray Beach, Florida, pp. 369-375.

M-3001 HANDLER, A. M. and GOMEZ, S. P. 1996. An Analysis of the *hobo* Transposable Element for Gene-Vector Development. In: B. A. McPheron and G. J. Steck. Fruit Fly Pests. St. Lucie Press, Delray Beach, Florida, pp. 221-229.

M-3002 GUPTA, P., DILLARD, C. R., and FERKOVICK, S. M. 1996 January. Potential of an Unnatural Host, *Galleria mellonella* (Lepidoptera: Galleriidae), in Rearing the Corn Earworm Endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 89 (1): 103-108.

M-3003 HEATH, R. R., EPSKY, N. D., JIMENEZ, A., DUEBEN, B. D., LANDOLT, P. J., MEYER, W. L., ALUJA, M., RIZZO, J., CAMINO, M., JERONIMO, F., and BARANOWSKI, R. M. 1996 March. Improved Pheromone-Based Trapping Systems to Monitor *Toxotrypana curvicauda* (Diptera: Tephritidae). Fla. Entomol. 79 (1): 37-48.

M-3004 WARD, K. E. and LANDOLT, P. J. 1995 November. Influence of Multiple Matings on Fecundity and Longevity of Female Cabbage Looper Moths (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 88 (6): 768-772.

M-3005 MACOM, T. E. and LANDOLT, P. J. 1995 December. *Elasmus polistis* (Hymenoptera: Eulophidae) Recovered from Nests of *Polistis dorsalis* (Hymenoptera: Vespidae) in Florida. Fla. Entomol. 78 (4): 612-614.

M-3006 GAZIT, Y., LEWIS, W. J., and TUMLINSON, J. H. 1996. Arrestment of *Telenomus remus* (Hymenoptera: Socelionisae) by a Kairomone Associated with Eggs of Its Host, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Biol. Control 6: 283-290.

M-3007 TEAL, P. E. A., ABERNATHY, R. L., NACCHMAN, R. J., FANG, N., MEREDITH, J. A., and TUMLINSON, J. H. 1996. Pheromone Biosynthesis Activating Neuropeptides: Functions and Chemistry. Peptides 17 (2): 337-344.

M-3008 SIVINSKI, J. M., CALKINS, C. O., BARANOWSKI, R., HARRIS, D., BRAMBILA, J., DIAZ, J., BURNS, R. E., HOLLER, T., and DODSON, G. 1996. Suppression of a Caribbean Fruit Fly (*Anastrepha suspensa* (Loew) Diptera: Tephritidae) Population through Augmented Releases of the Parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 6: 177-185.

M-3009 NACHMAN, R. J., RADEL, P. A., ABERNATHY, R., TEAL, P. E. A., HOLMAN, G. M., and YAMASHITA, O. 1995. Mimetic Analog Development for the Insect Pyrokinin/PBAN/Diapause Induction (FXPRLa) Neuropeptide Family. Molecular Mechanisms of Insect Metamorphosis and Diapause, pp. 97-106.

M-3010 EPSKY, N. D., HEATH, R. R., UCHIDA, G., GUZMAN, A., RIZZO, J., VARGAS, R., and JERONIMO, F. 1996 April. Capture of Mediterranean Fruit Flies (Diptera: Tephritidae) Using Color Inserts in Trimedlure-Baited Jackson Traps. Environ. Entomol. 25 (2): 256-260.

M-3011 TUMLINSON, J. H., TEAL, P. E. A., and FANG, N. 1996. The Integral Role of Triacyl Glycerols in the Biosynthesis of the Aldehydic Sex Pheromones of *Manduca sexta* (L.). Bioorganic & Medicinal Chemistry 4(3): 451-460.

M-3012 VALLES, S. M., YU, S. J., and KOEHLER, P. G. 1996 June. Biochemical Mechanisms Responsible for Stage-Dependent Propoxur Tolerance in the German Cockroach. Pesticide Biochem. Phsiol. 54: 172-180.

M-3013 FANG, N., TEAL, P. E. A., and TUMLINSON, J. H. 1996. Effects of Decapitation and PBAN Injection on Amounts of Triacylglycerols in the Sex Pheromone Gland of Manduca sexta (L.). Archives Insect Biochem. Physiol. 32: 249-260.

M-3014 KLOTZ, J. H., OI, D. H., VAIL, K. M., and WILLIAMS, D. F. 1996 June. Laboratory Evaluation of a Boric Acid Liquid Bait on Colonies of *Tapinoma melanocephalum*, Argentine Ants and Pharaoh Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 89: 673-677.

M-3015 MILLER, D. M., KOEHLER, P. G., and PATTERSON, R. S. 1996 June. Influence of German Cockroach (Dictyoptera: Blattellidae) Fecal Extract on Food Consumption and Harborage Choice. J. Econ. Entomol. 89: 668.

M-3016 VANDER MEER, R. K. 1996 July. Pheromone enhanced baits for pest ant control: current status and future prospects. Proc. of the 2nd International Conference on Insect Pests in the Urban Environment; Edinburgh, Scotland p. 531-539.

M-3017 WILLIAMS, D. F. and PORTER, S. D. 1996 April. Update on USDA-ARS Biological Control Studies on Fire Ants (Abstract). Proc. Of the 1996 Imported Fire Ant Conf., April 16-18, New Orleans, LA.

M-3018 JENSEN, T., COCKBURN, A. F., KAISER, P. E., and BARNARD, D. R. 1996 . Human Blood-Feeding Rates Among Sympatric Sibling Species of *Anopheles quadrimaculatus* Mosquitoes in Northern Florida. Am. J. Trop. Med. Hyg. 54 (5): 523-525.

M-3019 LECLERQ, S., BRAEKMAN, J. C., DALOZE, D., PASTEELS, J. M., and VANDER MEER, R. K. 1996 June. Biosynthesis of the Solenopsins, Venom Alkaloids of the Fire Ants. Naturwissenschaften 83: 222-225.

M-3020 KLOTZ, J. H. and WILLIAMS, D. F. 1996 August. New Approach to Boric Acid Ant Baits. IPM Practitioner 18 (8): 1-4.

M-3021 LANDOLT, P. J., MOLINA, O. H., HEATH, R. R., WARD, K. E., DUEBEN, B. D., and MILLAR, J. G. 1996 May. Starvation of Cabbage Looper Moths (Lepidoptera: Noctuidae) Increases Attraction to Male Pheromone. Ann. Entomol. Soc. Am. 89 (3): 459-465.

M-3022 HANDLER, A. M. and GOMEZ, S. P. 1996 July. The *hobo* Transposable Element Excises and has Related Elements in Tephritid Species. Genetics 143: 1339-1347.

M-3023 RÖSE, U. S. R., MANUKIAN, A., HEATH, R. R., and TUMLINSON J. H. 1996. Volatile Semiochemicals Released from Undamaged Cotton Leaves. Plant Physiol. 111: 487-495.

M-3024 FASULO, T. R. and KOEHLER, P. G. 1995. Computer-Verified Training Tutorials for Urban Pest Control Training. Journal of Extension 33 (2): 597-598.

M-3025 LANGE, C. E., BECNEL, J. J., RAZAFINDRATIANA, E., PRZYBYSZEWSKI, J., and RAZAFINDRAFARA, H. 1996 July. *Johenrea locustae* n.g., n.sp. (Microspora: Glugeidae): A Pathogen of Migratory Locusts (Orthoptera: Acrididae: Oedipodinae) from Madagascar. J. Invertebr. Pathol. 68: 28-40.

M-3026 REINERT, J. F., KAISER, P. E., and SEAWRIGHT, J. A. 1996 June. Unusual Features of Pupal Exuviae Noted in the *Anopheles quadrimaculatus* Complex (Diptera: Culicidae). J. Amer. Mosq. Control Assoc. 12 (2): 310-311.

M-3027 UNDEEN, A. H., and MOLLOY, D. P. 1996 June. Use of Stream Width for Determining the Dosage Rates of *Bacillus thuringiensis* var. *israelensis* for Larval Black Fly (Diptera: Simuliidae) Control. J. Amer. Mosq. Control Assoc. 12 (2): 312-315.

M-3028 MACOM, T. E., and PORTER, S. D. 1996 July. Comparison of Polygyne and Monogyne Red Imported Fire Ant (Hymenoptera: Formicidae) Population Densities. Ann. Entomol. Soc. Am. 89 (4): 535-543.

M-3029 GUPTA, P., SLOAN, A., DILLARD, C. R., and FERKOVICH, S. M. 1996 June. Parasitism of a Factitious Host, *Galleria mellonella* (Lepidoptera: Pyroalidae) by an Endoparasitoid: Oviposition and Emergence of *Microplitis croceipes* (Hymenoptera: Braconidae). Fla. Entomol. 79 (2): 221-229.

M-3030 HEATH, R. R., EPSKY, N. D., DUEBEN, B. D., and MEYER, W. L. 1996 June. Systems to Monitor and Suppress *Ceratitis capitata* (Diptera: Tephritidae) Populations. Fla. Entomol. 79 (2): 144-153.

M-3031 PARÉ, P. W., and TUMLINSON, J. H. 1996 June. Plant Volatile Signals in Response to Herbivore Feeding. Fla. Entomol. 79 (2): 93-103.

M-3032 HAEUSSNER, E. L., VANDER MEER, R. K., and CARDEILHAC, P. T. 1996 May. Effects of Alligator Egg Yolk Long-Chain Fatty Acids on Growth Performance of Hatchlings. Proc. of International Association for Aquatic Animal Medicine, Chattanooga, Tennessee; May 1996; p. 77-82.

M-3033 FOCKS, D. A. 1991 December. Data Sheet on Toxorhynchites. Division of Control of Tropical Disease, World Heatlh Organization, Geneva, Switzerland, 10 pp.; Vector Biology & Control Data Sheet.

M-3034 FOCKS, D. A. 1993 October. Avermectins in Medical and Veterinary Entomolgy. Current Status and Future Prospects (Abstract). Abst. 1st Int. Congr. Of Vector Ecology, San Diego, CA, Oct. 3-8, 1993.

M-3035 FOCKS, D. A. 1993. A Colour Atlas of Medical Entomology (Book Review). Parasitology Today 9: 478-479.

M-3036 OI, D. H., VAIL, K. M., and WILLIAMS, D. F. 1996 June. Field Evaluation of Perimeter Treatments for Pharaoh Ant (Hymenoptera: Formicidae) Control. Fla. Entomol. 79 (2): 252-263.

M-3037 HOGSETTE, J. A. 1996 August. Development of House Flies (Diptera: Muscidae) in Sand Containing Varying Amounts of Manure Solids and Moisture. J. Econ. Entomol. 89 (4): 940-945.

M-3038 GREENBERG-LEVY, S. H., ISHAAYA, I., SHAAYA, E., SILHACEK, D. L., and OBERLANDER, H. 1995. Hydrolase Activity in *Plodia interpunctella*: Use of Diflubenzuron and p-Nitroacetanilide as Substrates. Pesticide Biochem. and Physiol. 52: 157-169.

M-3039 VICK, K. W., LITZKOW, C. A., and SHUMAN, D. 1995 December. Acoustic Location Fixing Insect Detector. U. S. Patent 5,473,942.

M-3040 SHUMAN, D., WEAVER, D. K., MANKIN, R. W., CHIA, H., JOHNSON, R. E., NASAH-LIMA, M. N., RADFORD, R. R., SUN, J. S., and WEAVER, B. 1996 May. A Cross-Corrleation Implementation of the Acoustic Location Fingerprinting Insect Detector (ALFID) System. Proc. of the Second Symposium on Agroacoustics; Sept. 6-7, 1995, p.9-29.

M-3041 WEAVER, D. K., SHUMAN, D., MANKIN, R. W., JOHNSON, R. E., NASAH-LIMA, M. N., RADFORD, R. R., CHIA, H., and WEAVER, B. 1996 May. Performance of an Algorithm Based on Cross-Correlations that Detects the Number of Infested Wheat Kernels in Grain Samples Tested in ALFID. Proc. of the Second Symposium on Agroacoustics; September 6-7, 1995; p. 30-47.

M-3042 MANKIN, R. W., SUN, J. S., SHUMAN, D., and WEAVER, D. K. 1996 May. A Sound-Insulated Detectors from Grain Elevator Background Noise. Proc. of the Second Symposium on Agroacoustics; September 6-7, 1995; p. 89-104.

M-3043 SHUMAN, D. and NASAH-LIMA, N. 1996 July. A Distributed Multiplexing Transmission Network for Acquiring Data from Spatially Divergent Sensors. ASAE Annual International Meeting; July 16, 1996; p. 1-16.

M-3044 HAGSTRUM, D. W., FLINN, P. W., and SHUMAN, D. 1996 February. Automated Monitoring Using Acoustical Sensors for Insects in Farm-Stored Wheat. J. Econ. Entomol. 89 (1): 211-217.

M-3045 WEAVER, D. K., THRONE, J. E., BAKER, J. E., and CHEW, V. 1995. Potential "Probit-Type" Analyses for Rapid Assessment of Insecticide Resistance. Resistant Pest Management: A Biannual Newsletter; 7 (2): 29-31.

M-3046 THRONE, J. E., WEAVER, D. K., CHEW, V., and BAKER, J. E. 1995. Probit Analysis of Correlated Data: Multiple Observations Over Time at One Pesticide Concentration. Resistant Pest Management: A Biannual Newsletter; 7 (2): 20-21.

M-3047 HAILE, D. G., BIERY, T. L., and MOUNT, G. A. 1996 July. An Expert System for Aerial Pesticide Applications to Control Mosquitoes and Flies Using C-130 Aircraft. ASAE Annual Interantional Meeting; July 16, 1996; p.1-18.

M-3048 NACHMAN, R. J., TEAL, P. E. A., RADEL, P. A., HOMAN, G. M., and ABERNATHY, R. L. 1996. Potent Pheromonotropic/Myotropic Activity of a Carboranyl Pseudotetrapeptide Analogue of the Insect Pyrokinin/PBAN Neuropeptide Family Administered via Injection or Topical Application. Peptides 17 (5): 747-752.

M-3049 BERG, B. G., TUMLINSON, J. H., and MUSTAPARTA, H. 1995. Chemical Communication in Heliothine Moths iV. Receptor Neuron Responses to Pheromone Compounds in the Male Tobacco Budworm Moth *Heliothis virescens*. J. Comp. Physiol A 177: 527-534.

M-3050 HELM, R., COCKRELL, G., STANLEY, S., BRENNER, R. J., BURKS, W., and BANNON, G. 1996 July. Isolation and Characterization of a Clone Encoding a Major Allergen (Bla g 90kD) Involved in IgE Mediated Cockroach Hypersensitivity. J. Allergy Clin. Immunol. 98 (1): 172-180.

M-3051 VANDER MEER, R. K. and ALONSO, L. E. 1996 April. Fire Ant Alarm Pheromones. Proc. for the 1996 Imported Fire Ant Conf., April 16-18, 1996, New Orleans, LA.; p. 9-11 .

M-3053 PESQUERO, M. A., CAMPIOLO, S., FOWLER, H. G., and PORTER, S. D. 1996 September. Diurnal Patterns of Ovipositional Activity in Two Pseudacteon Fly Parasitoids (Diptera: Phoridae) of Solenopsis Fire Ants (Hymenoptera: Formicidae). Fla. Entomol. 79 (3): 455-457.

M-3054 WATSON, D. W., GEDEN, C. J., LONG, S. J., and RUTZ, D. A. 1995. Efficacy of *Beauveria bassiana* for the Control of House Fly and Stable Fly (Diptera: Muscidae). Biological Control 5: 405-411.

M-3055 GEDEN, C. J. 1996 June. Biological Control of Filth Flies. Symposium of Biological Control, June 9-14, 1996; 219-225.

M-3056 KLOTZ, J. H. and WILLIAMS, D. F. 1995 October. Ant Baits: A Case of Fatal Attraction. Pest Control : 82-84.

M-3057 FOWLER, H. G., PESQUERO, M. A., CAMPIOLO, S., ZACCARO, A., and PORTER, S. D. 1996 July. Formiga Perde a Cabeca Por Causa De Mosca. Ciencia Hoje 21 (122): 63-64.

M-3058 FASULO, T. R., KOEHLER, P. G., and GERBERG, E. J. 1996 February. An Urban Pest Knowledgebase. Fla. Coop. Ext. Ser./CD-ROM.

M-3059 LANDOLT, P. J., TOTH, M., FRANCKE, W., and MORI, K. 1996 September. Attraction of Moths (Lepidoptera: Acrtiidae, Geometridae, Noctuidae) to Enantiomers of Several Epoxydienes. Fla. Entomol. 79 (3): 392-397.

M-3060 PESQUERO, M. A., PORTER, S. D., FOWLER, H. G., and CAMPIOLO, S. 1995. Rearing of Pseudaction spp. (Diptera: Phoridae), Parasitoids of Fire Ants (Solenopsis spp.) (Hymenoptera: Formicidae). J. Appl. Entomol. 119: 677-678.

M-3061 GEDEN, C. J. 1996. Modeling Host Attacks and Progeny Production of *Spalangia gemina*, *Spalangia cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) at Constant and Variable Temperatures. Biological Control 7: 172-178.

M-3062 MOSER, B. A., BECNEL, J. J., UNDEEN, A. H., HODGE, N. C. and PATTERSON, R. S. 1996. Fatty Acid Analysis of Microsporidia. J. Invertebr. Pathol. 68: 146-151.

M-3063 PETITT, F. L., SHUMAN, D. S., WIETLISBACH, D. O., and COFFELT, J. A. 1996 September. An Automated System for Collection and Counting of Parasitized Leafminer (Diptera: Agromyzidae) Larvae. Fla. Entomol. 79 (3): 450-454.